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(54) RNA-CODED ANTIBODY

(57) The present application describes an antibody-coding, non-modified or modified RNA and the use thereof for expression of this antibody, for the preparation of a pharmaceutical composition, in particular a passive vaccine, for treatment of tumours and cancer diseases, cardiovascular diseases, infectious diseases, auto-immune diseases, virus diseases and monogenetic diseases, e.g. also in gene therapy. The present invention furthermore describes an in vitro transcription method, in vitro methods for expression of this antibody using the RNA according to the invention and an in vivo method.

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Description

[0001] The present application describes an antibody-coding, non-modified or modified RNA and the use thereof for expression of this antibody, for the preparation of a pharmaceutical composition, in particular a passive vaccine, for treatment of tumours and cancer diseases, cardiovascular diseases, infectious diseases, autoimmune diseases, virus diseases and monogenetic diseases, e.g. also in gene therapy. The present invention furthermore describes an in vitro transcription method, in vitro methods for expression of this antibody using the RNA according to the invention and an in vivo method.

[0002] The occurrence of tumours and cancer diseases is, alongside cardiovascular and infectious diseases, one of the most frequent causes of death in modern societies and is associated with usually considerable costs during the therapy and subsequent rehabilitation measures. The treatment of tumours and cancer diseases depends greatly, for example, on the nature of the tumour which occurs and at present conventionally is undertaken by using radio- or chemotherapy, in addition to invasive interventions. However, these therapies represent an exceptional burden on the immune system, and in some cases can be employed to only a limited extent. Furthermore, these therapy forms usually require long pauses between the individual treatments for regeneration of the immune system. In recent years, alongside these "conventional methods", molecular biology programmes in particular have emerged as promising for the treatment or for assisting these therapies.

[0003] An example of these molecular biology methods comprises the use of antibodies or immunoglobulins as essential effectors of the immune system. Antibodies or immunoglobulins can be generated either *in vitro* by using known molecular biology methods or by the immune system of the organism itself to be treated. The immune system of higher vertebrates thus has two separate functions of the immune system: the innate immune system, which reacts non-specifically to pathogens (e.g. by macrophage-mediated phagocytosis) and the adaptive immune system, which reacts specifically to pathogens by means of specialized effector cells (e.g. B and T cells). The antibodies or immunoglobulins which are secreted by plasma cells during an immune response are part of this adaptive immune system. Together with the complement system, they form the humoral branch of the immune response.

[0004] Alongside their essential importance for the immune system in higher vertebrates, precisely because of their high affinity and specificity for a particular antigen antibodies are an outstanding means both in biochemical and molecular biology research and in diagnostics and medical uses. Thus, antibodies are capable of binding specifically to their target structures (e.g. antigens, which substantially comprise proteins, peptides, in some cases lipids, carbohydrates etc.) and of thereby blocking (inhibiting) or, where appropriate, labelling these. They can moreover activate the immune system by means of their Fc part, so that the labelled cells are destroyed. Over 100 therapeutic antibodies are currently to be found in clinical studies. Antibodies which can be employed in cancer therapy play by far the greatest role in this context. Most of the antibodies prepared for this at present are monoclonal antibodies which originate originally, for example, from the mouse. In order to prevent an immune reaction against such monoclonal antibodies, at present chiefly humanized or human antibodies are employed for therapy (cf. David Male; "Immunologie auf einen Blick [Immunology at a Glance]", 1st German edition, 2005, Elsevier-Urban & Fischer Verlag; Charles A. Janeway, Paul Travers, Mark Walport and Mark Shlomchik, Immunobiology, 5th edition, 2001, Garland Publishing; Dissertation by Christian Klein, Monoklonale Antikörper und rekombinante Antikörperfragmente gegen sekundäre Arzneipflanzenmetabolite [Monoclonal Antibodies and Recombinant Antibody Fragments Against Secondary Medicinal Plant Metabolites], 2004; Andreas Schmiedl and Stefan Dübel, Rekombinante Antikörper & Phagen-Display [Recombinant Antibody & Phage Display], 2004, Molekulare Biotechnologie [Molecular Biotechnology] (Wiley-VCH)).

[0005] Antibodies generally can be assigned to the group of immunoglobulins. These immunoglobulins can in turn be differentiated into five main classes of immunoglobulins on the basis of their heavy chain, the IgM (μ), IgD (δ), IgG (y), IgA (α) and IgE (ε) antibodies, IgG antibodies making up the largest proportion. Immunoglobulins can moreover be differentiated into the isotypes κ and λ on the basis of their light chains.

[0006] In spite of their different specificity, antibodies are structurally quite similar in construction. Thus, IgG antibodies typically are built up two identical light and two heavy protein chains which are bonded to one another via disulfide bridges. The light chain comprises the N-terminal variable domain V_L and the C-terminal constant domain C_L. The heavy chain of an IgG antibody can be divided into an N-terminal variable domain V_H and three constant domains C_H1, C_H2 and C_H3 (cf. Fig. 1). While the amino acid sequence is largely the same in the region of the constant domains, wide differences in sequence are typically found within the variable domains.

[0007] The antibody repertoire of a human comprises about at least 10^{11} different antibody specificities. In higher vertebrates, the formation of antibodies takes place naturally in the immune system by somatic recombination. In this context, an organism is indeed theoretically capable of generating an antibody of appropriate specificity against any antigen. However, if each of these antibodies were to be coded by an endogenous gene, they would burst the human genome. Instead, in humans antibody genes are composed of a large number of individual gene segments. The part of the antibody gene which codes for the variable region of a light chain is formed from a V gene segment and a J gene segment. In this context, numerous different V and J segments are available, which can be combined with one another

virtually as desired. In this context, the variable region of a heavy chain is composed of three different gene segments. In addition to the V and J segments, additional D segments are also found here. The V_H, D_H and J_H segments can likewise be combined with one another virtually as desired to form the variable region of the heavy chain (cf. Fig. 2). The mechanism by which the various gene segments are combined to form complete antibody genes is called immunoglobulin rearrangement or somatic recombination. It takes place exclusively in B lymphocytes at certain times of cell development.

[0008] In addition to pure gene rearrangement, further mechanisms for increasing the antibody diversity also exist. Two mechanisms which are accompanied by somatic recombination are first to be mentioned in this context: The junctional diversity in this context describes controlled imprecise joining together of the rearranged gene segments, as a result of which random removal and insertion of nucleotides occurs at the cleavage sites. A further combinatorial diversity results from the possibility of combining a particular rearranged light chain with a particular rearranged heavy chain. Finally, the diversity of antibodies is also additionally increased after successful rearrangement and later activation of B cells, in that an affinity maturation of antibodies occurs due to an increased rate of mutation in the region of the variable regions of activated B cells (somatic hypermutation).

[0009] In addition to the formation of antibodies which takes place naturally by the immune system of the particular organism, antibodies can also be generated by molecular biology methods. However, in order to utilize the system elaborated for specification of antibody formation and specification thereof for particular antigens or nucleic acids, the formation of antibodies is at present typically induced in selected organisms by injection of a particular antigen, and the antibody is then isolated from the organism for further use. In this context, the B lymphocytes of the organism are conventionally purified selectively and fused with an immortal myeloma cell to form a hybridoma cell. Those cells which secrete the corresponding antigen-specific antibodies are then determined by selection methods.

[0010] In addition to use of hybridoma cells, recombinant preparation of these antibodies with the desired specificity is also possible after isolation and sequencing. Cells which provide the required posttranslational modifications are typically used for this. On the basis of the immune reaction with formation of human anti-mouse antibodies in the human organism in the case of native antibodies produced in the mouse (or in other hosts), chimeric, humanized or human antibodies are preferably prepared here.

[0011] After expression, these antibodies, optionally prepared by recombinant methods, can be employed as agents both in biochemical and molecular biology research, and in diagnostics and for medical uses.

[0012] In medical uses, however, in many cases antibodies can be employed directly only with difficulty, since these usually have only a very short half-life *in vivo* and therefore, possibly, cannot reach their target antigen or their target nucleic acid at all. This requires either high active compound concentrations of the desired antibody, or alternative methods which are suitable for providing large amounts of antibodies *in vivo*.

[0013] Such methods include, e.g. molecular medicine methods of gene therapy and genetic vaccination which, when used generally in the therapy and prevention of diseases, have considerable effects on medical practice. Both methods are based on the introduction of nucleic acids into cells or into tissue of the patient and on subsequent processing by the cells or, respectively, tissue of the information coded by the nucleic acids introduced, i.e. expression of the desired polypeptides, e.g. antibodies, in the cells or respectively, the tissue..

[0014] The conventional procedure of methods of gene therapy and of genetic vaccination to date is based on the use of DNA to sluice the required genetic information into the cell. Various methods for introducing DNA into cells have been developed in this connection, such as, for example, calcium phosphate transfection, polyprene transfection, protoplast fusion, electroporation, microinjection, lipofection and the use of gene canons, lipofection in particular having emerged as a suitable method.

[0015] A further method which has been proposed in particular in the case of genetic vaccination methods is the use of DNA viruses as DNA vehicles. Such viruses have the advantage that because of their infectious properties a very high transfection rate can be achieved. The viruses used are genetically modified, so that no functional infectious particles are formed in the transfected cell. The use of DNA viruses as DNA vehicles, however, has been criticized in recent years because of the risk of recombination of non-active viruses to give active viruses.

[0016] The use of DNA as an agent in gene therapy and genetic vaccination or for passive immunization (by passive vaccines), e.g. by using coding sequences for antibodies, may, however, also be less advantageous from some points of view. DNA is degraded only relatively slowly in the bloodstream, so that when (foreign) DNA is used as the coding sequence for a desired protein, a formation of anti-DNA antibodies may occur, which has been confirmed in an animal model in the mouse (Gilkeson et al., J. Clin. Invest. 1995, 95: 1398-1402). The possible persistence of (foreign) DNA in the organism can thus lead to a hyperactivation of the immune system, which as is known results in splenomegaly in mice (Montheith et al., Anticancer Drug Res. 1997, 12(5): 421-432). Furthermore, (foreign) DNA can interact with the host genome, and in particular cause mutations by integration into the host genome. Thus, for example, the (foreign) DNA introduced may be inserted into an intact gene, which represents a mutation which can impede or even completely switch off the function of the endogenous gene. On the one hand enzyme systems which are vital for the cell may be destroyed by such integration events, and on the other hand there is also the danger of a transformation of the cell

modified in this way into a degenerated state if a gene which is decisive for regulation of cell growth is modified by the integration of the foreign DNA. With the methods to date of gene therapy and genetic vaccination and also of passive immunization, a risk of development of cancer therefore cannot necessarily be ruled out when (foreign) DNA is used. In this context, passive immunization (by so-called "passive vaccines") is to be strictly differentiated from so-called active immunization. In active immunization, an antigen ("active vaccine") is typically administered, after which the organism forms antibodies against this antigen. Active immunization thus creates a permanent immunization of the organism against the particular antigen, which can be associated with the disadvantages described above. In passive immunization, in contrast, only an antiserum or the purified antibody itself ("passive vaccine") is administered to the organism. The coding sequence for the antibody can likewise be administered, as described above, as a so-called passive vaccine for passive immunization.

[0017] Summarizing, in the prior art there is an increased demand for and a considerable interest in agents which are suitable for employing antibodies effectively *in vivo,* in particular for providing increased active compound amounts of *antibodies in vivo,* without the risks hitherto associated with the use of DNA.

[0018] This object is achieved according to the invention by the use of an RNA (sequence) for intracellular expression of an antibody, wherein the RNA (sequence) codes for an antibody or contains at least one coding region, which codes for at least one antibody, respectively. In connection with the present invention, an antibody-coding RNA according to the invention includes any RNA which encodes an antibody. More generally, the RNA of the present invention (directed to intracellular expression) contains at least one coding region, wherein the at least one coding region codes for at least one antibody. If more than one coding region is contained in the RNA molecule of the invention, the second, third etc. coding region may code for antibodies as well, which may be the same or distinct from the first antibody coding region. In a preferred embodiment, the inventive RNA contains at least two coding regions, all of them coding for identical or distinct antibodies. In still another embodiment of the present invention, an inventive RNA may code for more than one antibody within the same coding region. In summary, the inventive RNA may be mono-, bi- or multicistronic, codes for at least one antibody.

[0019] The antibody-coding RNA according to the invention can be single-stranded or double-stranded, linear or circular, or in particular in the form of mRNA. The antibody-coding RNA according to the invention is particularly preferably in the form of single-stranded RNA, even more preferably in the form of mRNA.

[0020] An antibody-coding RNA according to the invention preferably has a length of from 50 to 15,000 nucleotides, more preferably a length of from 50 to 10,000 nucleotides, even more preferably a length of from 500 to 10,000 nucleotides and most preferably a length of from 500 to 7,000, 500 to 5,000 or 700 to 3,000 nucleotides.

[0021] In connection with the present invention, the antibodies coded by the RNA according to the invention can be chosen from all antibodies, e.g. from all antibodies which are generated by recombinant methods or are naturally occurring and are known to a person skilled in the art from the prior art, in particular antibodies which are (can be) employed for therapeutic purposes or for diagnostic or for research purposes or have been found with particular diseases, e.g. cancer diseases, infectious diseases etc.

[0022] In the context of the present invention, antibodies which are coded by an RNA according to the invention typically include all antibodies (described above) which are known to a person skilled in the art, e.g. naturally occurring antibodies or antibodies generated in a host organism by immunization, antibodies prepared by recombinant methods which have been isolated and identified from naturally occurring antibodies or antibodies generated in a host organism by (conventional) immunization or have been generated with the aid of molecular biology methods, as well as chimeric antibodies, human antibodies, humanized antibodies, bispecific antibodies, intrabodies, i.e. antibodies expressed in cells and possibly localized in particular cell compartments, and fragments of the abovementioned antibodies. Insofar, the term antibody is to be understood in its broadest meaning. In this context, antibodies in general typically comprise a light chain and a heavy chain, both of which have variable and constant domains. The light chain comprises the N-terminal variable domain V_L and the C-terminal constant domain C_L. The heavy chain of an IgG antibody, in contrast, can be divided into an N-terminal variable domain V_H and three constant domains C_H1, C_H2 and C_H3 (cf. Fig. 1).

[0023] RNA molecules according to the invention can also be prepared on the basis of polyclonal antibodies or, as an antibody-coding RNA cocktail, can have a polyclonal character. In the context of this invention, polyclonal antibodies are typically mixtures of antibodies against a specific antigen or immunogen or epitope of a protein which have been generated by immunization of a host organism, for example mammals, i.e. animals, including cattle, pigs, dogs, cats, donkeys, monkeys, including rodents, e.g. mice, hamsters, rabbits etc., and man. Polyclonal antibodies conventionally recognize different epitopes or regions of the same specific antigen, each of these epitopes in turn being capable of generating a clone of B lymphocytes which produces an antibody against this epitope. From such polyclonal antibodies or from the antibody sera obtained from the host organism, the individual antibodies specific against the particular epitopes can be obtained by individualization to monoclonal antibodies. The present invention accordingly also provides RNA which codes for a monoclonal antibody obtained by individualization of polyclonal antibodies.

[0024] Monoclonal antibodies in the context of this invention are therefore typically antibodies which are specific for a particular antigen or epitope (of a protein), i.e. bind this antigen or epitope (of a protein) with a high affinity, and

conventionally are expressed by a hybridoma cell. For the preparation of such monoclonal antibodies, the corresponding antigen or immunogen or epitope of a protein is typically injected at least once, but typically several times, into a host organism as described here, as a result of which the immune system of the host organism, in the presence of suitable adjuvants, is preferably stimulated to antibody production via activation of correspondingly specific B cells. The B lymphocytes are then conventionally selectively purified from the spleen or other organs or fluids suitable for this from an animal immunized in this manner, and are fused with an immortal myeloma cell to give the so-called hybridoma cell. After selection methods and cloning of the hybridomas or hybridoma cells formed, those clones which secemate, i.e. express and secrete, antibodies of the desired specificity can be determined. These clones can be isolated and sequenced with known molecular biology methods. The data obtained from such a sequencing can serve further in a nucleic acid synthesis for generation of synthetic DNA sequences or for screening a cDNA library and isolation of the cDNA fragments and generation of a DNA or nucleic acid template for *in vitro* or *in vivo* synthesis of the RNA according to the invention which codes for an antibody. Where appropriate, the RNA contained in the hybridomas can also be isolated, for example by fractionation, and subsequently the RNA molecules according to the invention which code for the hybridoma antibody can be purified by methods known to the person skilled in the art.

[0025] Nevertheless, RNA molecules which code for non-human monoclonal or polyclonal antibody, e.g. murine monoclonal antibodies or monoclonal antibodies from other, as described here, non-human host organisms or hybridoma cells are of only limited suitability for therapeutic use in humans, since in the human organism itself they conventionally cause an immune reaction with formation of human anti-antibodies directed against these non-human host antibodies. As a result, such non-human monoclonal or polyclonal antibodies as a rule can be administered to a person only a single time. To by-pass this problem, RNA molecules which code for chimeric, humanized and human antibodies can also be provided according to the invention.

[0026] Chimeric antibodies in the context of the present invention are preferably antibodies in which the constant domains of an antibody as described here have been replaced by human sequences. Preferably, chimeric antibodies are formed from monoclonal or polyclonal antibodies as described here.

[0027] Humanized antibodies in the context of the present invention are antibodies in which the constant and variable domains described above of the non-human monoclonal or polyclonal antibodies, with the exception of the hypervariable regions, have been replaced by human sequences.

[0028] RNA molecules which code for human antibodies, i.e. antibodies which have completely human sequences, that is to say in the constant and variable domains, including the hypervariable regions, can furthermore be used in the context of the present invention. Such RNA molecules which code for human antibodies can be isolated from human tissue or originate from immunized host organisms as described here, e.g. mice, which are then transgenic for the human IgG gene locus. RNA molecules which code for human antibodies and have been isolated by means of phage display and cloned with the aid of molecular biology methods are furthermore provided (see below).

[0029] Antibodies which are coded by RNAs according to the invention particularly preferably include so-called full length antibodies, i.e. antibodies which comprise both the complete heavy and the complete light chains, as described above.

[0030] RNAs which alternatively code for one or more antibody fragment(s) of the antibodies described above, instead of the corresponding full length antibody, can furthermore be provided in the context of the present invention. Examples of such antibody fragments are any antibody fragments known to a person skilled in the art, e.g. Fab, Fab', $F(ab')_2$, Fc, Facb, pFc', Fd, und Fv fragments of the abovementioned antibodies etc. A diagram of the structure of such antibody fragments is shown by way of example in Fig. 4. Protein fragments consisting of the minimal binding subunit of antibodies known as single-chain antibodies (scFvs) have excellent binding specificity and affinity for their ligands. In contrast to antibodies, scFvs lack the non-binding regions. Accordingly, RNA encoding scFvs are also encompassed by the present invention.

[0031] For example, an Fab (fragment antigen binding) fragment typically comprises the variable and a constant domain of a light and a heavy chain, e.g. the C_H1 and the V_H domain of the heavy chain and the complete light chain. The two chains are bonded to one another via a disulfide bridge. An Fab fragment thus conventionally contains the complete antigen-binding region of the original antibody and usually has the same affinity for the antigen, the immunogen or an epitope of a protein. Antibody fragments, as also described above for antibodies, can be prepared with the aid of molecular biology methods. In this context, the DNA sequences which code for the various domains of the antibody fragment are cloned into a specific expression vector. The RNA which codes for these antibody fragments can then be expressed e.g. in suitable host cells. Suitable host cells in connection with the present invention include, inter alia, *E. coli*, yeasts, transgenic plants or mammalian cells etc. (see below). In contrast, an scFv fragment (single chain variable fragment) typically comprises the variable domain of the light and of the heavy chain, which are bonded to one another via an artificial polypeptide linker. In the cloning of such scFv fragments, RNAs which code for a V_H and V_L, these being linked to one another by a polypeptide linker, are preferably provided. As a rule, a polypeptide built up from 15-25 glycine, proline and/or serine residues (cf. Fig. 5) or the associated nucleotide sequence is used at the RNA level for the provision of this component.

[0032] Furthermore, RNA molecules which code for bispecific antibodies can also be provided in the context of the present invention. Bispecific antibodies in the context of the present invention are preferably antibodies which can act as adaptors between an effector and a corresponding target, e.g. for recruiting effector molecules (e.g. toxins, active compounds (drugs), cytokines etc.), targeting of effector cells (e.g. CTL, NK cells, macrophages, granulocytes etc. (see, for example, review by Kontermann R.E., Acta Pharmacol. Sin, 2005, 26(1): 1-9). In this context, bispecific antibodies are in principle built up such as is described here in general for antibodies, these bispecific antibodies e.g. recognizing two different antigens, immunogens or epitopes, or active compounds, cells, or other molecules (or structures) as mentioned above, i.e. the antigen-binding regions of the antibody are specific for two different molecules (or structures). The various antigens, immunogens or epitopes etc., for example, can thus be brought spatially close. Furthermore, by the binding e.g. of a binding domain or other specificities, the function of the antibody can be extended specifically, e.g. of a binding protein, an immunotoxin etc. Such bispecific antibodies can also be single-chain antibodies (e.g. scFv fragments etc.). Bispecific antibodies can be used, for example, to bring two reaction partners, e.g. two cells, two proteins, a protein and the substrate thereof etc., spatially close in order to promote an interaction between these (e.g. protein-protein interactions, substrate conversions, modifications etc.). Bispecific antibodies are used above all to bring effector cells (such as, for example, T cells, NK cells, macrophages etc.) and target cells (e.g. tumour cells, infected cells etc.) spatially close. Examples of bispecific antibodies can include, without being limited thereto, e.g. those antibodies or antibody fragments which bind on the one hand a surface factor as described here, and on the other hand an antigen as described here, preferably a tumour antigen as described here. This includes e.g. CD28 and a tumour antigen (Grosse-Hovest L. et al., 2003, Eur. Immunol. 33(5); 1334-40, (A recombinant bispecific single-chain antibody induces targeted, supra-agonistic CD28-stimulation and tumor cell killing)), CD 19 and CD3 (CD19 tumour antigen of B cell lymphoma) etc.

[0033] Without being limited thereto, according to the present invention RNAs which code for antibodies inter alia code for those antibodies which bind antigens or specific nucleic acids. Antigens in the context of the present invention are typically molecules which are recognized as exogenous by the immune system and conventionally cause an immune reaction or immune response with the formation of antibodies directed specifically against them. However, antigens can also include, especially in the case of autoimmune diseases, endogenous molecules or structures which are incorrectly recognized as exogenous by the immune system and thereby trigger an immune reaction. Alternatively formulated, antigens are therefore all molecules which are recognized by an antibody in the context of the present invention. Antigens substantially comprise proteins, peptides or epitopes of these proteins or peptides. In this context, epitopes (also called "antigenic determinants") are typically small regions (molecular sections) lying on the surface of such protein or peptide structures and having a length of from 5 to 15, in rare case also to 25, preferably 6 to 9 amino acids. Antigens can furthermore also include lipids, carbohydrates etc. In the context of the present invention, antigens also include, for example, so-called immunogens, i.e. antigens which lead to an immunity of the organism transfected therewith. Antigens by way of example include, without being limited thereto, surface antigens of cells, tumour antigens etc. For example, according to the present invention antibodies can bind the following antigens (which typically occur in vertebrates), e.g. tumour-specific surface antigens (TSSA), e.g. 5T4, $\alpha 5\beta 1$-integrin, 707-AP, AFP, ART-4, B7H4, BAGE, β-catenin/m, Bcr-abl, MN/C IX-antigen, CA125, CAMEL, CAP-1, CASP-8, CD4, CD 19, CD20, CD22, CD25, CDC27/m, CD 30, CD33, CD52, CD56, CD80, CDK4/m, CEA, CT, Cyp-B, DAM, EGFR, ErbB3, ELF2M, EMMPRIN, EpCam, ETV6-AML1, G250, GAGE, GnT-V, Gp100, HAGE, HER-2/neu, HLA-A*0201-R170I, HPV-E7, HSP70-2M, HAST-2, hTERT (or hTRT), iCE, IGF-1R, IL-2R, IL-5, KIAA0205, LAGE, LDLR/FUT, MAGE, MART-1/Melan-A, MART-2/Ski, MC1R, myosin/m, MUC1, MUM-1, -2, -3, NA88-A, NY-ESO1, PAP, proteinase-3, p190 minor bcr-abl, Pml/RARα, PRAME, PSA, PSM, PSMA, RAGE, RU1 or RU2, SAGE, SART-1 or SART-3, survivin, TEL/AML1, TGFβ, TPI/m, TRP-1, TRP-2, TRP-2/INT2, VEGF and WT1, or sequences, such as e.g. NY-Eso-1 or NY-Eso-B. Tumour antigens can, for example, typically be responsible for metastasing, that is to say dissolving of tumour cells out of their native tissue, transfer into the vascular system (lymph or blood vessel system), exit from the vascular system and colonization in a new tissue. In this context, such tumour antigens which cause modified cell-cell interactions compared with the native state are of interest in particular.

[0034] Antibodies encoded by the inventive RNA may also be directed against tumour antigens listed by Table 1 or Table 2. In particular, RNA encoding those antibodies may be used to treat (or, may be used to prepare a medicament to treat, respectively) the cancer diseases given in the last column of Tables 1 and 2.

Table 1: Antigens expressed in cancer diseases

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
5T4		colorectal cancer, gastric cancer, ovarian cancer
707-AP	707 alanine proline	melanoma
9D7		renal cell carcinoma

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
AFP	alpha-fetoprotein	hepatocellular carcinoma, gallbladder cancer, testicular cancer, ovarian cancer, bladder cancer
AlbZIP HPG1		prostate cancer
alpha5beta1-Integrin		
alpha5beta6-Integrin		colon cancer
alpha-methylacyl-coenzyme A racemase		prostate cancer
ART-4	adenocarcinoma antigen recognized by T cells 4	lung cancer, head and neck cancer, leukemia, esophageal cancer, gastric cancer, cervical cancer, ovarian cancer, breast cancer, squamous cell carcinoma
B7H4		ovarian cancer
BAGE-1	B antigen	bladder cancer, head and neck cancer, lung cancer, melanoma, squamous cell carcinoma
BCL-2		leukemia
BING-4		melanoma
CA 15-3/CA 27-29		breast cancer, ovary cancer, lung cancer, prostate cancer
CA 19-9		gastric cancer, pancreatic cancer, liver cancer, breast cancer, gallbladder cancer, colon cancer, ovary cancer, lung cancer
CA 72-4		ovarian cancer
CA125		ovarian cancer, colorectal cancer, gastric cancer, liver cancer, pancreatic cancer, uterus cancer, cervix carcinoma, colon cancer, breast cancer, lung cancer
calreticulin		bladder cancer
CAMEL	CTL-recognized antigen on melanoma	melanoma
CASP-8	caspase-8	head and neck cancer
cathepsin B		breast cancer
cathepsin L		breast cancer
CD19		B-cell malignancies
CD20		
CD22		
CD25		
CD30		
CD33		
CD4		
CD52		
CD55		

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
CD56		
CD80		
CEA	carcinoembryonic antigen	gut carcinoma, colorectal cancer, colon cancer, hepatocellular cancer, lung cancer, breast cancer, thyroid cancer, pancreatic cancer, liver cancer cervix cancer, bladder cancer, melanoma
CLCA2	calcium-activated chloride channel-2	lung cancer
CML28		leukemia
Coactosin-like protein		pancreatic cancer
Collagen XXIII		prostate cancer
COX-2		ovarian cancer, breast cancer, colorectal cancer
CT-9/BRD6	bromodomain testis-specific protein	
Cten	C-terminal tensin-like protein	prostate cancer
cyclin B1		
cyclin D1		ovarian cancer
cyp-B	cyclophilin B	bladder cancer, lung cancer, T-cell leukemia, squamous cell carcinoma,
CYPB1	cytochrom P450 1B1	leukemia
DAM-10/MAGE-B1	differentiation antigen melanoma 10	melanoma, skin tumors, ovarian cancer, lung cancer
"DAM-6/MAGE-B2	differentiation antigen melanoma 6	melanoma, skin tumors, ovarian cancer, lung cancer
EGFR/Her1		lung cancer, ovarian cancer, head and neck cancer, colon cancer, pancreatic cancer, breast cancer
EMMPRIN	tumor cell-associated extracellular matrix metalloproteinase inducer/	lung cancer, breast cancer, bladder cancer, ovarian cancer, brain cancer, lymphoma
EpCam	epithelial cell adhesion molecule	ovarian cancer, breast cancer, colon cancer, lung cancer
EphA2	ephrin type-A receptor 2	glioma
EphA3	ephrin type-A receptor 2	melanoma, sarcoma, lung cancer
ErbB3		breast cancer
EZH2	(enhancer of Zeste homolog 2)	endometrium cancer, melanoma, prostate cancer, breast cancer
FGF-5	fibroblast growth factor-5	renal cell carcinoma, breast cancer, prostate cancer
FN	fibronectin	melanoma
Fra-1	Fos-related antigen-1	breast cancer, esophageal cancer, renal cell carcinoma, thyroid cancer
G250/CAIX	glycoprotein 250	leukemia, renal cell carcinoma, head and neck cancer, colon cancer, ovarian cancer, cervical cancer
GAGE-1	G antigen 1	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
GAGE-2	G antigen 2	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-3	G antigen 3	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-4	G antigen 4	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-5	G antigen 5	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-6	G antigen 6	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-7b	G antigen 7b	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GAGE-8	G antigen 8	bladder cancer, lung cancer, sarcoma, melanoma, head and neck cancer
GDEP	gene differentially expressed in prostate	prostate cancer
GnT-V	N-acetylglucosaminyltransferase V	glioma, melanoma
gr100	glycoprotein 100 kDa	melanoma
GPC3	glypican 3	hepatocellular carcinoma, melanoma
HAGE	helicase antigen	bladder cancer
HAST-2	human signet ring tumor-2	
hepsin		prostate
Her2/neu/ErbB2	human epidermal receptor-2/neurological	breast cancer, bladder cancer, melanoma, ovarian cancer, pancreas cancer, gastric cancer
HERV-K-MEL		melanoma
HNE	human neutrophil elastase	leukemia
homeobox NKX 3.1		prostate cancer
HOM-TES-14/SCP-1		ovarian cancer
HOM-TES-85		
HPV-E6		cervical cancer
HPV-E7		cervical cancer
HST-2		gastric cancer
hTERT	human telomerase reverse transcriptase	breast cancer, melanoma, lung cancer, ovarian cancer, sarcoma, Non-Hodgkin-lymphoma, acute leukemia
iCE	intestinal carboxyl esterase	renal cell carcinoma
IGF-1R		colorectal cancer
IL-13Ra2	interleukin 13 receptor alpha 2 chain	glioblastoma
IL-2R		colorectal cancer
IL-5		

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
immature laminin receptor		renal cell carcinoma
kallikrein 2		prostate cancer
kallikrein 4		prostate cancer
Ki67		prostate cancer, breast cancer, Non-Hodgkin-lymphoma, melanoma
KIAA0205		bladder cancer
KK-LC-1	Kita-kyushu lung cancer antigen 1	lung cancer
KM-HN-1		tongue cancer, hepatocellular carcinomas, melanoma, gastric cancer, esophageal, colon cancer, pancreatic cancer
LAGE-1	L antigen	bladder cancer, head and neck cancer, melanoma
livin		bladder cancer, melanoma
MAGE-A1	melanoma antigen-A1	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A10	melanoma antigen-A10	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A12	melanoma antigen-A12	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia, prostate cancer, myeloma, brain tumors
MAGE-A2	melanoma antigen-A2	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A3	melanoma antigen-A3	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A4	melanoma antigen-A4	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A6	melanoma antigen-A6	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-A9	melanoma-antigen-A9	bladder cancer, head and neck cancer, melanoma, colon cancer, lung cancer, sarcoma, leukemia
MAGE-B1	melanoma-antigen-B1	melanoma
MAGE-B10	melanoma-antigen-B10	melanoma
MAGE-B16	melanoma-antigen-B16	melanoma
MAGE-B17	melanoma-antigen-B17	melanoma
MAGE-B2	melanoma-antigen-B2	melanoma
MAGE-B3	melanoma-antigen-B3	melanoma
MAGE-B4	melanoma-antigen-B4	melanoma
MAGE-B5	melanoma-antigen-B5	melanoma
MAGE-B6	melanoma-antigen-B6	melanoma
MAGE-C1	melanoma-antigen-C1	bladder cancer, melanoma
MAGE-C2	melanoma-antigen-C2	melanoma
MAGE-C3	melanoma-antigen-C3	melanoma

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
MAGE-D1	melanoma-antigen-D1	melanoma
MAGE-D2	melanoma-antigen-D2	melanoma
MAGE-D4	melanoma-antigen-D4	melanoma
MAGE-E1	melanoma-antigen-E1	bladder cancer, melanoma
MAGE-E2	melanoma-antigen-E2	melanoma
MAGE-F1	melanoma-antigen-F1	melanoma
MAGE-H1	melanoma-antigen-H1	melanoma
MAGEL2	MAGE-like 2	melanoma
mammaglobin A		breast cancer
MART-1/Melan-A	melanoma antigen recognized by T cells-1/melanoma antigen A	melanoma
MART-2	melanoma antigen recognized by T cells-2	melanoma
matrix protein 22		bladder cancer
MC1R	melanocortin 1 receptor	melanoma
M-CSF	macrophage colony-stimulating factor gene	ovarian cancer
mesothelin		ovarian cancer
MG50/PXDN		breast cancer, glioblastoma, melanoma
MMP 11	M-phase phosphoprotein 11	leukemia
MN/CA IX-antigen		renal cell carcinoma
MRP-3	multidrug resistance-associated protein 3	lung cancer
MUC1	mucin 1	breast cancer
MUC2	mucin 2	breast cancer, ovarian cancer, pancreatic cancer
NA88-A	NA cDNA clone of patient M88	melanoma
N-acetylglucos-aminyltransferase-V		
Neo-PAP	Neo-poly(A) polymerase	
NGEP		prostate cancer
NMP22		bladder cancer
NPM/ALK	nucleophosmin/anaplastic lymphoma kinase fusion protein	
NSE	neuron-specific enolase	small cell cancer of lung, neuroblastoma, Wilm' tumor, melanoma, thyroid cancer, kidney cancer, testicle cancer, pancreas cancer
NY-ESO-1	New York esophageous 1	bladder cancer, head and neck cancer, melanoma, sarcoma, B-lymphoma, hepatoma, pancreatic cancer, ovarian cancer, breast cancer
NY-ESO-B		
OA1	ocular albinism type 1 protein	melanoma

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
OFA-iLRP	oncofetal antigen-immature laminin receptor	leukemia
OGT	O-linked N-acetylglucosamine trans-ferase gene	
OS-9		
osteocalcin		prostate cancer
osteopontin		prostate cancer, breast cancer, ovarian cancer
p15	protein 15	
P15		melanoma
p190 minor bcr-abl		
p53		
PAGE-4	prostate GAGE-like protein-4	prostate cancer
PAI-1	plasminogen acitvator inhibitor 1	breast cancer
PAI-2	plasminogen acitvator inhibitor 2	breast cancer
PAP	prostate acic phosphatase	prostate cancer
PART-1		prostate cancer
PATE		prostate cancer
PDEF		prostate cancer
Pim-1-Kinase		
Pin1	Propyl isomerase	prostate cancer
POTE		prostate cancer
PRAME	preferentially expressed antigen of melanoma	melanoma, lung cancer, leukemia, head and neck cancer, renal cell carcinoma, sarcoma
prostein		prostate cancer
proteinase-3		
PSA	prostate-specific antigen	prostate cancer
PSCA		prostate cancer
PSGR		prostate cancer
PSM		
PSMA	prostate-specific membrane antigen	prostate cancer
RAGE-1	renal antigen	bladder cancer, renal cancer, sarcoma, colon cancer
RHAMM/CD168	receptor for hyaluronic acid mediated motility	leukemia
RU1	renal ubiquitous 1	bladder cancer, melanoma, renal cancer
RU2	renal ubiquitous 1	bladder cancer, melanoma, sarcoma, brain tumor, esophagel cancer, renal cancer, colon cancer, breast cancer
S-100		melanoma
SAGE	sarcoma antigen	

(continued)

Tumor antigen	Name of tumor antigen	Cancers or cancer diseases related thereto
SART-1	squamous antigen rejecting tumor 1	esophageal cancer, head and neck cancer, lung cancer, uterine cancer
SART-2	squamous antigen rejecting tumor 1	head and neck cancer, lung cancer, renal cell carcinoma, melanoma, brain tumor
SART-3	squamous antigen rejecting tumor 1	head and neck cancer, lung cancer, leukemia, melanoma, esophageal cancer
SCC	squamous cell carcinoma antigen	lung cancer
Sp17	sperm protein 17	multiple myeloma
SSX-1	synovial sarcoma X breakpoint 1	hepatocellular cell carcinom, breast cancer
SSX-2/HOM-MEL-40	sarcoma X breakpoint 2	breast cancer
SSX-4	synovial sarcoma X breakpoint 4	bladder cancer, hepatocellular cell carcinoma, breast cancer
STAMP-1		prostate cancer
STEAP	six transmembrane epithelial antigen prostate	prostate cancer
survivin		bladder cancer
survivin-2B	intron 2-retaining survivin	bladder cancer
TA-90		melanoma
TAG-72		prostate carcinoma
TARP		prostate cancer
TGFb	TGFbeta	
TGFbRII	TGFbeta receptor II	
TGM-4	prostate-specific transglutaminase	prostate cancer
TRAG-3	taxol resistant associated protein 3	breast cancer, leukemia, and melanoma
TRG	testin-related gene	
TRP-1	tyrosine related protein 1	melanoma
TRP-2/6b	TRP-2/novel exon 6b	melanoma, glioblastoma
TRP-2/INT2	TRP-2/intron 2	melanoma, glioblastoma
Trp-p8		prostate cancer
Tyrosinase		melanoma
UPA	urokinase-type plasminogen activator	breast cancer
VEGF	vascular endothelial growth factor	
VEGFR-2/FLK-1	vascular endothelial growth factor receptor-2	
WT1	Wilm' tumor gene	gastric cancer, colon cancer, lung cancer, breast cancer, ovarian cancer, leukemia

Table 2: Mutant antigens expressed in cancer diseases

Mutant antigen	Name of mutant antigen	Cancers or cancer diseases related thereto
alpha-actinin-4/m		lung carcinoma
ARTC1/m		melanoma
bcr/abl	breakpoint cluster region-Abelson fusion protein	CML
beta-Catenin/m	beta-Catenin	melanoma
BRCA1/m		breast cancer
BRCA2/m		breast cancer
CASP-5/m		colorectal cancer, gastric cancer, endometrial carcinoma
CASP-8/m		head and neck cancer, squamous cell carcinoma
CDC27/m	cell-division-cycle 27	
CDK4/m	cyclin-dependent kinase 4	melanoma
CDKN2A/m		melanoma
CML66		CML
COA-1/m		colorectal cancer
DEK-CAN	fusion protein	AML
EFTUD2/m		melanoma
ELF2/m	Elongation factor 2	lung squamous cell carcinoma
ETV6-AML1	Ets variant gene6/acute myeloid leukemia 1 gene fusion protein	ALL
FN1/m	fibronectin 1	melanoma
GPNMB/m		melanoma
HLA-A*0201-R170I	arginine to isoleucine exchange at residue 170 of the alpha-helix of the alpha2-domain in the HLA-A2 gene	renal cell carcinoma
HLA-A11/m		melanoma
HLA-A2/m		renal cell carcinoma
HSP70-2M	heat shock protein 70-2 mutated	renal cell carcinoma, melanoma, neuroblastoma
KIAA0205/m		bladder tumor
K-Ras/m		pancreatic carcinoma, colorectal carcinoma
LDLR-FUT	LDR-Fucosyltransferase fusion protein	melanoma
MART2/m		melanoma
ME1/m		non-small cell lung carcinoma
MUM-1/m	melanoma ubiquitous mutated 1	melanoma
MUM-2/m	melanoma ubiquitous mutated 2	melanoma
MUM-3/m	melanoma ubiquitous mutated 3	melanoma
Myosin class I/m		melanoma
neo-PAP/m		melanoma

(continued)

Mutant antigen	Name of mutant antigen	Cancers or cancer diseases related thereto
NFYC/m		lung squamous cell carcinoma
N-Ras/m		melanoma
OGT/m		colorectal carcinoma
OS-9/m		melanoma
p53/m		
Pml/RARa	promyelocytic leukemia/retinoic acid receptor alpha	APL, PML
PRDX5/m		melanoma
PTPRK/m	receptor-type protein-tyrosine phosphatase kappa	melanoma
RBAF600/m		melanoma
SIRT2/m		melanoma
SYT-SSX-1	synaptotagmin I/synovial sarcoma X fusion protein	sarcoma
SYT-SSX-2	synaptotagmin I/synovial sarcoma X fusion protein	sarcoma
TEL-AML1	translocation Ets-family leukemia/acute myeloid leukemia 1 fusion protein	AML
TGFbRII	TGFbeta receptor II	colorectal carcinoma
TPI/m	triosephosphate isomerase	melanoma

[0035] In a preferred embodiment according to the invention, antibodies encoded by the inventive RNA are directed against the following (protein) antigens (whereby the RNA molecules may be used for the preparation of a medicament, e.g. a pharmaceutical composition or more preferably a (passive) vaccine in the meaning of the present inventino), are selected from the group consisting of 5T4, 707-AP, 9D7, AFP, AlbZIP HPG1, alpha-5-beta-1-integrin, alpha-5-beta-6-integrin, alpha-actinin-4/m, alpha-methylacyl-coenzyme A racemase, ART-4, ARTC1/m, B7H4, BAGE-1, BCL-2, bcr/abl, beta-catenin/m, BING-4, BRCA1/m, BRCA2/m, CA 15-3/CA 27-29, CA 19-9, CA72-4, CA125, calreticulin, CAMEL, CASP-8/m, cathepsin B, cathepsin L, CD19, CD20, CD22, CD25, CDE30, CD33, CD4, CD52, CD55, CD56, CD80, CDC27/m, CDK4/m, CDKN2A/m, CEA, CLCA2, CML28, CML66, COA-1/m, coactosin-like protein, collage XXIII, COX-2, CT-9/BRD6, Cten, cyclin B1, cyclin D1, cyp-B, CYPB1, DAM-10, DAM-6, DEK-CAN, EFTUD2/m, EGFR, ELF2/m, EMMPRIN, EpCam, EphA2, EphA3, ErbB3, ETV6-AML1, EZH2, FGF-5, FN, Frau-1, G250, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE7b, GAGE-8, GDEP, GnT-V, gp100, GPC3, GPNMB/m, HAGE, HAST-2, hepsin, Her2/neu, HERV-K-MEL, HLA-A*0201-R17I, HLA-A11/m, HLA-A2/m, HNE, homeobox NKX3.1, HOM-TES-14/SCP-1, HOM-TES-85, HPV-E6, HPV-E7, HSP70-2M, HST-2, hTERT, iCE, IGF-1R, IL-13Ra2, IL-2R, IL-5, immature laminin receptor, kallikrein-2, kallikrein-4, Ki67, KIAA0205, KIAA0205/m, KK-LC-1, K-Ras/m, LAGE-Al, LDLR-FUT, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-A10, MAGE-A12, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E2, MAGE-F1, MAGE-HI, MAGEL2, mammaglobin A, MART-1/melan-A, MART-2, MART-2/m, matrix protein 22, MC1R, M-CSF, ME1/m, mesothelin, MG50/PXDN, MMP11, MN/CA IX-antigen, MRP-3, MUC-1, MUC-2, MUM-1/m, MUM-2/m, MUM-3/m, myosin class I/m, NA88-A, N-acetylglucosaminyltransferase-V, Neo-PAP, Neo-PAP/m, NFYC/m, NGEP, NMP22, NPM/ALK, N-Ras/m, NSE, NY-ESO-1, NY-ESO-B, OA1, OFA-iLRP, OGT, OGT/m, OS-9, OS-9/m, osteocalcin, oste-opontin, p15, p190 minor bcr-abl, p53, p53/m, PAGE-4, PAI-1, PAI-2, PART-1, PATE, PDEF, Pim-1-Kinase, Pin-1, Pml/PARalpha, POTE, PRAME, PRDX5/m, prostein, proteinase-3, PSA, PSCA, PSGR, PSM, PSMA, PTPRK/m, RAGE-1, RBAF600/m, RHAMM/CD168, RU1, RU2, S-100, SAGE, SART-1, SART-2, SART-3, SCC, SIRT2/m, Sp17, SSX-1, SSX-2/HOM-MEL-40, SSX-4, STAMP-1, STEAP, survivin, survivin-2B, SYT-SSX-1, SYT-SSX-2, TA-90, TAG-72, TARP, TEL-AML1, TGFbeta, TGFbetaRII, TGM-4, TPI/m, TRAG-3, TRG, TRP-1, TRP-2/6b, TRP/INT2, TRP-p8, tyrosinase, UPA, VEGF, VEGFR-2/FLK-1, and WT1.

[0036] In a particularly preferred embodiment, the RNA codes for antibodies which are directed against protein antigens selected from the group consisting of MAGE-A1, MAGE-A6, melan-A, GP100, tyrosinase, survivin, CEA, Her-2/neu, WT1, PRAME, EGFRI (epidermal growth factor receptor 1), mucin-1 and SEC61G, hTERT, 5T4, NY-Esol, and TRP-2, more preferably from sequences of group consisting of MAGE-A1 [accession number M77481], MAGE-A6 [accession

number NM_005363], melan-A [accession number NM_005511], GP100 [accession number M77348], tyrosinase [accession number NM_000372], survivin [accession number AF077350], CEA [accession number NM_004363], Her-2/neu [accession number M11730], WT1 [accession number NM_000378], PRAME [accession number NM_006115], EGFRI (epidermal growth factor receptor 1) [accession number AF288738], mucin-1 [accession number NM_002456] and SEC61G [accession number NM_014302], hTERT [accession number NM_198253], 5T4 [accession number NM_006670], NY-Esol [accession number NM_001327], and TRP-2 [accession number NM_001922].

[0037] Antibodies (and therefore also the RNAs according to the invention on which these antibodies are based) which bind the antigens described here and, possibly, other antigens or nucleic acids can be identified e.g. by means of the method of phage display developed by George P. Smith. In this context, antibodies or antibody fragments are typically expressed on the surface of filamentous phages (Smith, G.P., 1985, "Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface", Science 228; 1315-1317). For this there are conventionally 3 to 5 copies of the surface protein gpIII on the proximal end of the phage, with the aid of which the phage infects bacteria cells via the F pilus thereof. In phage display, for example, the DNA for an antibody fragment which codes the antigen-binding variable domain is then cloned in-frame before the gpIII gene. In protein biosynthesis, a fusion protein is formed therefrom, which is expressed on the virus surface without the phage losing its infectiousness. With the aid of the phage display technique, it is possible to generate large antibody libraries, each phage expressing a different antibody fragment on the surface. To this extent, the underlying RNA is therefore also available. A particular antibody fragment can be isolated from such a library by a method called "phage panning". For this, the corresponding antigen is bound to a matrix and incubated with the phage suspension. The phages which present an appropriate antibody fragment interact with the fixed antigen, while the other phages are removed by a washing step. The phages isolated are multiplied, for example, in *E. coli*. The DNA is isolated accordingly and the gene sequence is determined. Expression constructs which contain the cDNA coding for the entire antibody or antibody fragments can then be developed with the aid of genetic engineering methods. An RNA (mRNA) which codes for the antibody can be generated from this cDNA by means of *in vitro* transcription (see below). Nucleic acids or, respectively, mRNA coding for monoclonal antibodies which are entirely of human origin are obtained in this manner.

[0038] In the context of the present invention, RNA according to the invention which codes for antibodies as described above is also suitable for coding so-called intrabodies or for rendering possible an expression of intrabodies. Intrabodies in the context of the present invention can include any of the antibodies or antibody fragments described here. Intrabodies are intracellularly expressed antibodies, i.e. antibodies which are coded by nucleic acids localized in the cell and are expressed there. For this, an RNA according to the invention which encodes the antibodies or antibody fragments as described above is introduced into cells beforehand, for example with the aid of transfection methods according to the invention or other suitable transfection methods (see below) and, where appropriate, thereafter transplanted into an organism or being or introduced directly as nucleic acids into an organism or being. In this context (irrespective of whether an an intrabody or a secreted antibody shall be introduce into the cell), the RNA according to the invention (or a corresponding nucleic acid) can be introduced in the naked form or as a complex with suitable carriers (e.g. liposomes) into the organism or the being or can have such modifications (of the RNA) which, where appropriate together with one of the transfection methods mentioned, lead to a better cell uptake, e.g. any of the RNA modifications mentioned here, such as, for example, lipid modifications of the RNA according to the invention. An organism or a being in connection with the present invention typically means mammals, i.e. animals, including cattle, pig, dog, cat, donkey, monkey, rodents, e.g. mouse, hamster, rabbit etc., and humans. Intrabodies can be localized and expressed at certain sites in the cell. For example, intrabodies can be expressed in the cytoplasm, the formation of disulfide bridges usually being decreased under the reducing conditions of the cytoplasm. It has been possible to demonstrate, however, that cytoplasmic intrabodies, and in particular scFv fragments, can be functional. Cytoplasmic expression by the RNA according to the invention opens up the possibility of also inhibiting cytoplasmic proteins. This is not possible with treatment with monoclonal antibodies from the prior art, since these antibodies can reach only secreted and membrane-located (extracellular) proteins due to their secretion from the cell after intracellular expression (which represents the major difference between antibodies and intrabodies). By expression of a signal peptide, intrabodies can be transported into the endoplasmic reticulum (ER) and then secreted as with regular antibodies. In this case, typically only secreted or membrane-located proteins are a target for these antibodies. By additional coding of a C-terminal ER retention signal (for example KDEL) by the RNA according to the invention, the intrabody can remain in the ER (where it may bind to specific antigen located in the ER) and prevent secretion of its antigen and/or transport of its antigen or its target molecule to the plasma membrane. Depending on the requirement, intrabodies can include full length antibodies or antibody fragments as described above. Intrabodies in the context of the present invention preferably initially include full length antibodies, which are retained in the cell and not secreted from the cell (by whatever technique, e.g. retention signal sequences etc.). However, if e.g. intracellular expression of full length antibodies is technically not possible or not appropriate, antibody fragments as described above can also be employed as intrabodies.

[0039] Antibodies which are coded by the RNA according to the invention furthermore also include those antibodies or antibody fragments which have a sequence identity to one of the antibodies or antibody fragments described here of

at least 70 %, 80% or 85 %, preferably at least 90 %, more preferably at least 95 % and most preferably at least 99 % over the entire length of the coding nucleic acid or amino acid sequence of an antibody or antibody fragment as described here. Preferably, such antibodies or antibody fragments have the same biological function as or, respectively, the specific activity of the corresponding full length antibody, e.g. the specific binding of particular antigens or nucleic acids. Accordingly, it is preferred, if the hypervariable region(s) are conserved or are modified by merely conservative mutations.

[0040] The biological function of antibodies described here which are coded by the RNA according to the invention includes e.g. neutralization of antigens, complement activation or opsonization. In the case of neutralization of antigens, the antibody can bind to an antigen and thereby neutralize this. The antibody is conventionally blocked by the binding of the antigen, and can therefore display its action only against one antigen, or two antigens in the case of bispecific antibodies. scFv antibody fragments are suitable above all for this (neutralization) function of an antibody, since they do not include the functions of the constant domains of an antibody. In the case of complement activation, the complex system of complement proteins which are dependent upon the Fc part of the antibody can be activated via binding of antibodies. End products of the complement cascade typically lead to lysis of cells and to the creation of a phlogistic (inflammatory) milieu. In the case of opsonization, pathogens or foreign particles are rendered accessible to phagocytes by binding by an antibody via the constant domains of the antibody. Alternatively, the opsonized cells, which are recognized as foreign, can be lysed via an antibody-dependent, cell-mediated cytotoxicity (ADCC). In this context, NK cells in particular can perform lytic functions in this manner via activation of their Fc receptors.

[0041] In connection with the present invention, the term "identity" means that the sequences are compared with one another as follows. In order to determine the percentage identity of two nucleic acid sequences, the sequences can first be aligned with respect to one another in order subsequently to make a comparison of these sequences possible. For this e.g. gaps can be inserted into the sequence of the first nucleic acid sequence and the nucleotides can be compared with the corresponding position of the second nucleic acid sequence. If a position in the first nucleic acid sequence is occupied by the same nucleotide as is the case at a position in the second sequence, the two sequences are identical at this position. The percentage identity between two sequences is a function of the number of identical positions divided by the number of all the positions compared in the sequences investigated. If e.g. a specific sequence identity is assumed for a particular nucleic acid (e.g. a nucleic acid which codes for a protein, as described above) in comparison with a reference nucleic acid (e.g. a nucleic acid from the prior art) of defined length, this percentage identity is stated relatively with reference to this reference nucleic acid. Starting therefore, for example, from a nucleic acid which has a sequence identity of 50 % to a reference nucleic acid 100 nucleotides long, this nucleic acid can be a nucleic acid 50 nucleotides long which is completely identical to a 50 nucleotides long section of the reference nucleic acid. Indeed, it can also be a nucleic acid 100 nucleotides long which has 50 % identity, i.e. in this case 50 % identical nucleic acids, with the reference nucleic acid over the entire length thereof. Alternatively, this nucleic acid can be a nucleic acid 200 nucleotides long which is completely identical in a 100 nucleotides long section of the nucleic acid to the reference nucleic acid 100 nucleotides long. Other nucleic acids of course equally meet these criteria. The identity statements described for nucleic acids apply equally to the antibodies and antibody fragments coded by the RNA according to the invention. The same holds for the determination of the sequence identity between two (poly)peptides, based on the comparison/alignment of the respective amino acid sequences.

[0042] The percentage identity of two sequences can be determined with the aid of a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used for comparison of two sequences is the algorithm of Karlin et al. (1993), PNAS USA, 90:5873-5877. Such an algorithm is integrated in the NBLAST program, with which sequences which have a desired identity to the sequences of the present invention can be identified. In order to obtain a gapped alignment, as described here, the "Gapped BLAST" program can be used, as is described in Altschul et al. (1997), Nucleic Acids Res, 25:3389-3402. If BLAST and Gapped BLAST programs are used, the preset parameters of the particular program (e.g. NBLAST) can be used. The sequences can be aligned further using version 9 of GAP (global alignment program) of the "Genetic Computing Group" using the preset (BLOSUM62) matrix (values -4 to +11) with a gap open penalty of -12 (for the first zero of a gap) and a gap extension penalty of -4 (for each additional successive zero in the gap). After the alignment, the percentage identity is calculated by expressing the number of agreements as a percentage content of the nucleic acids in the sequence claimed. The methods described for determination of the percentage identity of two nucleic acid sequences can also be used correspondingly, if necessary, on the coded amino acid sequences, e.g. the antibodies described here.

[0043] According to a preferred embodiment, the antibody-coding RNA according to the invention contains acoding regions which codes for one of the anribodies listed in Table 3. The antibody encoding RNA may be used to treat (or, may be used to provide a pharmaceutical composition to treat) one of the diseases, disorders, pathologies listed in the right-hand column of Table 3.

Table 3

Name	Target	Clinical application
Oregovomab (OvaRex)	CA125 (MUC-16)	Ovarian Cancer, Fallopian Tube Cancer, Peritoneal Cavity Cancer
Cantuzumab	CanAg (MUC-1)	Colon Cancer, Gastric Cancer, Pancreatic Cancer, NSCLC
HuC242-DM4	CanAg (MUC-1)	Colon Cancer, Gastric Cancer, Pancreatic Cancer
PAM4 (IMMU-107)	CanAg (MUC-1)	Pancreatic Cancer
HuC242-DM4	CanAg (MUC-1)	Colorectal Cancer; Pancreatic Cancer
HuHMFG1	CanAg (MUC-1)	Breast Cancer
WX-G250 (Rencarex)	Carbonische Anhydrase IX (G250)	Renal Cell Carcinoma
MT103	CD19	Non-Hodgkin-Lymphoma
Ibritumomab (Zevalin)	CD20	Non-Hodgkin-Lymphoma, Lymphoma
Rituximab (Rituxan, MabThera)	CD20	Non-Hodgkin-Lymphoma, Lymphoma, Chronic Lymphocytic Leukemia
Tositumomab (Bexxar)	CD20	Non-Hodgkin-Lymphoma, Lymphoma, Myeloma
Ofatumamab (HuMax-CD20)	CD20	Lymphoma, B-Cell Chronic Lymphocytic Leukemia
Epratuzumab (LymphoCide)	CD22	Non-Hodgkin-Lymphoma, Leukemia
MDX-060	CD30	Hodgkin-Lymphoma, Lymphoma
SGN-30	CD30	Hodgkin-Lymphoma, Lymphoma
Gemtuzumab (Mylotarg)	CD33	Leukemia
Zanolimumab (HuMax-CD4)	CD4	T-Cell-Lymphoma
SGN-40	CD40	Non-Hodgkin-Lymphoma, Myeloma, Leukemia, Chronic Lymphocytic Leukemia
Alemtuzumab (MabCampath)	CD52	T-Cell-Lymphoma, Leukemia
HuN901-DM1	CD56	Myeloma
Galiximab	CD80	Non-Hodgkin-Lymphoma
Labetuzumab	CEA	Colon Cancer, Pancreatic Cancer, Ovarian Cancer
Ipilimumab (MDX-010)	CTLA4	Sarcoma, Melanoma, Lung cancer, Ovarian Cancer leucemia, Lymphoma, Brain and Central Nervous System Tumors, Testicular Cancer, Prostate Cancer, Pancreatic Cancer, Breast Cancer

(continued)

Name	Target	Clinical application
Cetuximab (Erbitux)	EGFR	Colon Cancer, Head and Neck Cancer, Pancreatic Cancer, Non-Small Cell Lung Cancer, Cervical Cancer, Endometrial Cancer, Breast Cancer, Myeloma, Lung Cancer, Gastric Cancer, Esophageal Cancer, Pancreatic Cancer, Oropharyngeal Neoplasms, Hepatocellular Carcinoma, Squamous Cell Carcinoma, Sarcoma, Larynx Cancer; Hypopharynx Cancer
Panitumumab (Vectibix)	EGFR	Colon Cancer, Lung Cancer, Breast Cancer; Bladder Cancer; Ovarian Cancer
Nimotuzumab (TheraCim)	EGFR	Solid Tumors, Lung Cancer
Matuzumab	EGFR	Lung Cancer, Cervical Cancer, Esophageal Cancer
Zalutumumab	EGFR	Head and Neck Cancer, Squamous Cell Cancer
Pertuzumab (Omnitarg)	EGFR und HER2/neu	Breast Cancer, Ovarian Cancer, Lung Cancer, Prostate Cancer
Catumaxomab (Removab)	EpCam	Ovarian Cancer, Fallopian Tube Neoplasms, Peritoneal Neoplasms
MORab-003	GP-3	Ovarian Cancer, Fallopian Tube Cancer, Peritoneal Cancer
MORab-009	GP-9	Pancreatic Cancer, Mesothelioma, Ovarian Cancer, Non-Small Cell Lung Cancer, Fallopian Tube Cancer, Peritoneal Cavity Cancer
Ertumaxomab	HER2/neu	Breast Cancer
Trastuzumab (Herceptin))	HER2/neu	Breast Cancer, Endometrial Cancer, Solid Tumors
AMG 102	HGF	Advanced Renal Cell Carcinoma
Apolizumab (Remitogen)	HLA-DR-Antigen	Solid Tumors, Leukemia, Non-Hodgkin-Lymphoma, Lymphoma
CNTO 95	Integrin-Rezeptor	Melanoma
ID09C3	MHCII	Non-Hodgkin-Lymphoma
Denosumab (AMG-102)	RANKL	Myeloma, Giant Cell Tumor of Bone, Breast Cancer, Prostate Cancer
GC1008	TGFbeta	Advanced Renal Cell Carcinoma; Malignant Melanoma
Mapatumumab	TRAIL-R1	Colon Cancer, Myeloma
Bevacizumab (Avastin)	VEGF	Colon Cancer, Breast Cancer, Brain and Central Nervous System Tumors, Lung Cancer, Hepatocellular Carcinoma, Kidney Cancer, Breast Cancer, Pancreatic Cancer, Bladder Cancer, Sarcoma, Melanoma, Esophageal Cancer; Stomach Cancer, Metastatic Renal Cell Carcinoma; Kidney Cancer, Glioblastoma, Liver Cancer
MEDI 522	VLA3 (alpha5beta3-Integrin)	Solid Tumors, Leukemia, Lymphoma, Small Intestine Cancer, Melanoma
Volociximab	VLA5 (alpha5betal-Integrin)	Renal Cell Carcinoma, Pancreatic Cancer, Melanoma
Hematology:		
Name	**Target**	**Application**
Eculizumab (Alexion)	C5 Komplementfaktor	Paroxysmale nächtliche Hämoglobinurie (PNH)

(continued)

Hematology:		
Name	**Target**	**Application**
Mepolizumab	Interleukin-5	Hypereosinophilie-Syndrom
Dentology:		
Name	**Target**	**Application**
CaroRx (CaroRx)	Streptococcus mutans	Zahnkaries
Autoimmune Diseases und allergic Diseases:		
Name	**Target**	**Application**
Efalizumab (Raptiva)	CD11a	Psoriasis (Schuppenflechte)
Epratuzumab (LymphoCide)	CD22	Autoimmune Diseases, Non-Hodgkin-Lymphom
Lumiliximab	CD23	Allergies
Daclizumab	CD25	Schubförmige Multiple Sclerosis
Natalizumab (Tysabri)	CD49d	Multiple Sclerosis
Omalizumab (Xolair)	IgE (Fc-Teil)	Schweres Asthma bronchiale
Mepolizumab	Interleukin-5	Asthma, Hypereosinophilic Syndrome, Eosinophilic Gastroenteritis, Churg-Strauss Syndrome, Eosinophilic Esophagitis
Tocilizumab (Actemra)	Interleukin-6	Rheumatoid Arthritis
Adalimumab (Humira)	TNFα	Rheumatoid Arthritis, Psoriasis-Arthritis, Morbus Bechterew
Infliximab (Remicade)	TNFα	Morbus Crohn, Rheumatoide Arthritis, Morbus Bechterew, Psoriasis-Arthritis, Colitis ulcerosa, Psoriasis (Schuppenflechte)
Golimumab (CNTO 148)	TNFα	Rheumatoid Arthritis
Mapatumumab	TRAIL-R1	Myeloma
Rituximab (Rituxan, MabThera)	CD20	Urticaria, Rheumatoid Arthritis, Ulcerative Colitis, Chronic Focal Encephalitis
Epratuzumab (LymphoCide)	CD22	Autoimmune diseases, Systemic Lupus Erythematosus
Neurodegenerative Diseases:		
Name	**Target**	**Application**
R1450	Amyloid-beta	Alzheimer
Ophthalmology:		
Name	**Target**	**Application**
Ranibizumab (Lucentis)	VEGF-A	Feuchte Macular Degeneration

(continued)

Ophthalmology:		
Name	**Target**	**Application**
Bevacizumab (Avastin)	VEGF	Macular Degeneration
Infektious Diseases:		
Name	**Target**	**Application**
Palivizumab (Synagis)	Component of RSV (Respiratory Syncytial Virus)	Prevention of RSV-Pneumonia bei Fruhgeborenen
Cardiovascular Diseases:		
Name	**Target**	**Application**
Abciximab (ReoPro)	GPIIb/Iia	Verhinderung eines Gefäßverschlusses nach PTCA
Other Diseases:		
Name	**Target Application**	
Denosumab (AMG-102)	RANKL Osteoporosis	
GC1008	TGFbeta Pulmonary Fibrosis	
Bevacizumab (Avastin)	VEGF Proliferative Diabetic Retinopathy	

[0044] According to a preferred embodiment, the antibody-coding RNA according to the invention contains or has a sequence which codes for the heavy chains according to SEQ ID NO: 2 and the light chains according to SEQ ID NO: 4. According to an even more preferred embodiment, the antibody-coding RNA according to the invention contains or has a coding sequence according to SEQ ID NO: 5 or SEQ ID NO: 51, respectively.

[0045] According to another preferred embodiment, the antibody-coding RNA according to the invention contains or has a sequence which codes for the heavy chains according to SEQ ID NO: 7 and the light chains according to SEQ ID NO: 9. According to an even more preferred embodiment, the antibody-coding RNA according to the invention contains or has a coding sequence according to SEQ ID NO: 10 or SEQ ID NO: 52, respectively.

[0046] According to a further preferred embodiment, the antibody-coding RNA according to the invention contains or has a sequence which codes for the heavy chains according to SEQ ID NO: 12 and the light chains according to SEQ ID NO: 14. According to an even more preferred embodiment, the antibody-coding RNA according to the invention contains or has a coding sequence according to SEQ ID NO: 15 or SEQ ID NO: 53, respectively.

[0047] Antibodies which are coded by the RNA according to the invention can furthermore also encode such antibodies which have a sequence identity to one of the coding sequences of the antibodies described here, e.g. as described by Table 3 or by SEQ ID NO: 5 (51), 10 (52) or 15 (53), of at least 70 %, 80 % or 85 %, preferably at least 90 %, more preferably at least 95 % and most preferably at least 99 % over the entire length of the nucleic acid sequence or amino acid sequence of an antibody as described here, e.g. as described by Table 3 or by SEQ ID NO: 5 (51), 10 (52) or 15 (53).

[0048] Such antibodies which are coded by the RNA according to the invention likewise include antibodies according to SEQ ID NO: 5 (51), 10 (52) or 15 (53) or according to Table 3 which contain or have, in one of the heavy chains described here according to SEQ ID NO: 2, 7 or 12 and/or in one of the light chains described here according to SEQ ID NO: 4, 9 or 14, a nucleic acid or amino acid sequence identity of at least 70 %, 80 % or 85 %, preferably at least 90 %, more preferably at least 95 % and most preferably at least 99 % over the entire length of the coding sequence for the particular light and/or heavy chain, with an otherwise unchanged coding antibody sequence of SEQ ID NO: 5 (51), 10 (52) or 15 (53) or e.g antibodies of Table 3.

[0049] Overall, a novel route for carrying out antibody therapies on the basis of RNA, in particular mRNA, is thus provided with the aid of the present invention. In such a manner, clinically tested antibodies, for example angiogenesis inhibitors based on antibodies, for example bevacizumab (monoclonal immunoglobulin G_1 antibody which binds to the vascular growth factor VEGF (vascular endothelial growth factor); or trastuzumab (Herceptin), an indirect inhibitor which inhibits the action of tumour proteins on receptors, or for example rituximab or cetuximab (directed against the epidermal

growth factor receptor (EGFR)), based on RNA, can be provided, so that the inventive RNA contains at least one coding region which codes for at least one of these antibodies.

[0050] In a preferred embodiment, the antibody-coding RNA according to the invention typically additionally has at least one of the following modifications, which are preferably suitable for increasing the stability of the coding RNA, improving the expression of the antibody thereby coded, increasing the cell permeability, rendering possible localization of the antibody on or in certain cell compartments etc. Each of these modifications of the RNA according to the invention described here (modified RNA) which are mentioned in the following can be combined with one another in a suitable manner, such modifications which do not interfere with one another or adversely influence the stability or cell permeability of the antibody-coding, modified RNA according to the invention or the expression of the antibody thereby coded preferably being combined with one another. For the entire present invention, the nomenclature "modified" is equated with the content of "optionally modified".

[0051] Modifications of the RNA according to the invention described here (modified RNA) can include, for example, modifications of the nucleotides of the RNA. An RNA (modified RNA) according to the invention can thus include, for example, backbone modifications, sugar modifications or base modifications. In this context, the antibody-coding RNA according to the invention typically first contains nucleotides which can be chosen from all naturally occurring nucleotides and analogues thereof (modified nucleotides), such as e.g. ribonucleotides and/or deoxyribonucleotides. Nucleotides in the context of the present invention therefore include, without being limited thereto, for example purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, β-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art e.g. from the US patents 4,373,071, US 4,401,796, US 4,415,732, US 4,458,066, US 4,500,707, US 4,668,777, US 4,973,679, US 5,047,524, US 5,132,418, US 5,153,319, US 5,262,530 and 5,700,642, the disclosure of which is included here in its full scope by reference.

[0052] In particular, an antibody-coding RNA according to the invention can contain RNA backbone modifications. In connection with the present invention, a backbone modification is a modification in which the phosphates of the backbone of the nucleotides contained in the RNA are modified chemically. In this context, such backbone modifications typically include, without being limited thereto, modifications from the group consisting of methylphosphonates, methylphosphoramidates, phosphoramidates, phosphorothioates (e.g. cytidine 5'-O-(1-thiophosphate)), boranophosphates, positively charged guanidinium groups etc., which means by replacing the phosphodiester linkage by other anionic, cationic or neutral groups.

[0053] An antibody-coding RNA according to the invention can likewise also contain sugar modifications. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides it contains and typically includes, without being limited thereto, sugar modifications chosen from the group consisting of 2'-deoxy-2'-fluoro-oligoribonucleotide (2'-fluoro-2'-deoxycytidine 5'-triphosphate, 2'-fluoro-2'-deoxyuridine 5'-triphosphate), 2'-deoxy-2'-deamine-oligoribonucleotide (2'-amino-2'-deoxycytidine 5'-triphosphate, 2'-amino-2'-deoxyuridine 5'-triphosphate), 2'-O-alkyloligoribonucleotide, 2'-deoxy-2'-C-alkyloligoribonucleotide (2'-O-methylcytidine 5'-triphosphate, 2'-methyluridine 5'-triphosphate), 2'-C-alkyloligoribonucleotide, and isomers thereof (2'-aracytidine 5'-triphosphate, 2'-arauridine 5'-triphosphate), or azidotriphosphates (2'-azido-2'-deoxycytidine 5'-triphosphate, 2'-azido-2'-deoxyuridine 5'-triphosphate).

[0054] Preferably, however, the modified RNA sequence according to the invention contains no sugar modifications or backbone modifications if e.g. an *in vitro* transcription is necessary. The reason for this preferred exclusion lies in the problem that certain backbone modifications and sugar modifications of RNA sequences on the one hand can prevent or at least greatly reduce *in vitro* transcription thereof. Thus, an *in vitro* transcription of eGFP carried out by way of example functions, for example, only with the sugar modifications 2'-amino-2'-deoxyuridine 5'-phosphate, 2'-fluoro-2'-deoxyuridine 5'-phosphate and 2'-azido-2'-deoxyuridine 5'-phosphate. In addition, the translation of the protein, i.e. the protein expression, *in vitro* or *in vivo* typically can be reduced considerably by backbone modifications and, independently thereof, by sugar modifications of RNA sequences. It was possible to demonstrate this, for example, for eGFP in connection with the backbone modifications and sugar modifications selected above.

[0055] An antibody-coding RNA according to the invention can likewise also contain modifications of the bases of the nucleotides it contains (base modifications). Thus, for example, the antibody-coding RNA according to the invention can be modified such that only one or several of the nucleotides of the modified RNA are exchanged for nucleotides having

base modifications, which are preferably suitable for increasing the expression of the antibody coded by the RNA significantly compared with the non-modified, i.e. native RNA sequence. In this case, significant means an increase in the expression of the antibody on the basis of the modified RNA sequence compared with the native RNA sequence by at least 20 %, preferably at least 30 %, 40 %, 50 % or 60 %, even more preferably by at least 70 %, 80 %, 90 % or even 100 % and most preferably by at least 150 %, 200 % or even 300 %. In connection with the present invention, a modified nucleotide which contains a base modification is called a base-modified nucleotide and, without being limited thereto, is preferably chosen from the group consisting of: 2-amino-6-chloropurine riboside 5'-triphosphate, 2-aminoadenosine 5'-triphosphate, 2-thiocytidine 5'-triphosphate, 2-thiouridine 5'-triphosphate, 4-thiouridine 5'-triphosphate, 5-aminoallylcytidine 5'-triphosphate, 5-aminoallyluridine 5'-triphosphate, 5-bromocytidine 5'-triphosphate, 5-bromouridine 5'-triphosphate, 5-iodocytidine 5'-triphosphate, 5-iodouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate, 5-methyluridine 5'-triphosphate, 6-azacytidine 5'-triphosphate, 6-azauridine 5'-triphosphate, 6-chloropurine riboside 5'-triphosphate, 7-deazaadenosine 5'-triphosphate, 7-deazaguanosine 5'-triphosphate, 8-azaadenosine 5'-triphosphate, 8-azidoadenosine 5'-triphosphate, benzimidazole riboside 5'-triphosphate, N1-methyladenosine 5'-triphosphate, N1-methylguanosine 5'-triphosphate, N6-methyladenosine 5'-triphosphate, O6-methylguanosine 5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate. Nucleotides for base modifications are particularly preferably chosen from the group of base-modified nucleotides consisting of 5-methylcytidine 5'-triphosphate and pseudouridine 5'-triphosphate.

[0056] Without being restricted thereto, in this connections the inventors attribute an increase in the expression of the antibody coded by the (base)-modified RNA according to the invention to, inter alia, the improvement in the stabilizing of secondary structures and, where appropriate, to the "more rigid" structure formed in the RNA and the increased base stacking. Thus, for example, it is known of pseudouridine 5'-triphosphate that this occurs naturally in structural RNAs (tRNA, rRNA and snRNA) in eukaryotes as well as in pro-karyotes. In this connection, it is assumed that pseudouridine is necessary in rRNA for stabilizing secondary structures. In the course of evolution, the content of pseudouridine in RNA has increased, and it has been possible to demonstrate, surprisingly, that the translation depends on the presence of pseudouridine in the tRNA and rRNA, the interaction between tRNA and mRNA presumably being intensified in this context. The conversion of uridine into pseudouridine takes place posttranscriptionally by pseudouridine synthase. A posttranscriptional modification of RNA likewise takes place in the case of 5-methylcytidine 5'-triphosphate, and is catalysed by methyltransferases. A further increase in the content of pseudouridine and the base modification of other nucleotides is assumed to lead to similar effects, which, in contrast to the naturally occurring increased contents of pseudouridine in the sequence, can be carried out in a targeted manner and with a considerably wider variability. For 5-methylcytidine 5'-triphosphate and the further base modifications mentioned here, a similar mechanism to that for pseudouridine 5'-triphosphate is therefore assumed, i.e. an improved stabilizing of secondary structures, and on the basis of this an improved translation efficiency. In addition to this structurally based increase in expression, however, a positive effect on the translation is presumed, independently of the stabilizing of secondary structures and a "more rigid" structure of the RNA. Further causes of the increase in expression are also to be found, possibly, in the lower degradation rate of the RNA sequences by RNAses *in vitro* or *in vivo.*

[0057] The modifications of the antibody-coding RNA according to the invention which are described above can be introduced into the RNA with the aid of methods known to a person skilled in the art. Possible methods for this are, for example, synthesis methods using (automatic or semi-automatic) oligonucleotide synthesis apparatuses, biochemical methods, such as e.g. *in vitro* transcription methods, etc. Preferably, in this connection, for (shorter) sequences which in general do not exceed a length of 50-100 nucleotides, synthesis methods using (automatic or semi-automatic) oligonucleotide synthesis apparatuses and also *in vitro* transcription methods can be employed. For (longer) sequences, e.g. sequences which have a length of more than 50 to 100 nucleotides, biochemical methods are preferred, such as, for example, *in vitro* transcription methods, preferably an *in vitro* transcription method as described here, optionally using the modified RNA according to the invention.

[0058] Modifications with nucleotides as described here in an antibody-coding RNA according to the invention can occur on at least one (modifiable) nucleotide of the RNA sequence according to the invention, preferably on at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 (modifiable) nucleotides, more preferably on at least 10-20 (modifiable) nucleotides, even more preferably on at least 10-100 (modifiable) nucleotides and most preferably on at least 10-200, 10 to 1,000 or 10 to 10,000 or more (modifiable), e.g. all, nucleotides. Worded alternatively, modifications in an antibody-coding RNA according to the invention can occur on at least one (modifiable) nucleotide of the RNA sequence according to the invention, preferably on at least 10 % of all the (modifiable) nucleotides, more preferably on at least 25 % of all the (modifiable) nucleotides, even more preferably on at least 50 % of all the (modifiable) nucleotides, even more preferably on at least 75 % of all the (modifiable) nucleotides and most preferably on 100 % of the (modifiable) nucleotides contained in the RNA sequence according to the invention. In this connection, a "modifiable nucleotide" is any (preferably naturally occurring (native) and therefore non-modified) nucleotide which is to be exchanged for a nucleotide modified as described here. In this context, all the nucleotides of the RNA sequence can be modified, or only certain selected nucleotides of the RNA sequence. If all the nucleotides of the RNA sequence are to be modified, 100 % of the "modifiable nucleotides" of the

RNA sequence are all the nucleotides of the RNA sequence used. On the other hand, if only certain selected nucleotides of the RNA sequence are to be modified, the selected nucleotides are, for example, adenosine, cytidine, guanosine or uridine. Thus, for example, an adenosine of the native sequence can be exchanged for a modified adenosine, a cytidine for a modified cytidine, a uridine for a modified uridine and a guanosine for a modified guanosine. In this case, 100 % of the "modifiable nucleotides" of the RNA sequence are 100 % of the adenosines, cytidines, guanosines and/or uridines in the RNA sequence used.

[0059] According to another very preferred embodiment of the present invention, the antibody-coding RNA according to the invention can contain, for example, a GC content which has been modified compared with the native, i.e. non-modified (precursor) RNA sequence. According to a first alternative of the antibody-coding RNA according to the invention, the G/C content for the coding region of the RNA according to the invention is greater than the G/C content for the coding region of the native RNA sequence, the coded amino acid sequence of the antibody or antibody fragment being unchanged compared with the wild-type, i.e. the antibody or antibody fragment amino acid sequence coded by the native RNA sequence. In this context, the composition and the sequence of the various nucleotides plays a major role. In particular, sequences having an increased G (guanine)/C (cytosine) content are more stable than sequences having an increased A (adenine)/U (uracil) content. According to the invention, the codons are therefore varied compared with the wild-type RNA, while retaining the translated amino acid sequence, such that they include an increased amount of G/C nucleotides. Since several codons code for one and the same amino acid (degeneration of the genetic code), the most favourable codons for the stability can be determined (alternative codon usage).

[0060] Depending on the amino acid to be coded by the antibody-coding RNA according to the invention, there are various possibilities for modification of the native sequence of the RNA according to the invention. In the case of amino acids which are coded by codons which contain exclusively G or C nucleotides, no modification of the codon is necessary. Thus, the codons for Pro (CCC or CCG), Arg (CGC or CGG), Ala (GCC or GCG) and Gly (GGC or GGG) require no modification, since no A or U is present.

[0061] In the following cases, the codons which contain A and/or U nucleotides are modified by substitution of other codons which encode the same amino acids but contain no A and/or U. Examples are:

- the codons for Pro can be modified from CCU or CCA to CCC or CCG;
- the codons for Arg can be modified from CGU or CGA or AGA or AGG to CGC or CGG;
- the codons for Ala can be modified from GCU or GCA to GCC or GCG;
- the codons for Gly can be modified from GGU or GGA to GGC or GGG.

[0062] In other cases, although A or U nucleotides cannot be eliminated from the codons, it is however possible to decrease the A and U content by using codons which contain fewer A and/or U nucleotides. For example:

- the codons for Phe can be modified from UUU to UUC;
- the codons for Leu can be modified from UUA, CUU or CUA to CUC or CUG;
- the codons for Ser can be modified from UCU or UCA or AGU to UCC, UCG or AGC;
- the codon for Tyr can be modified from UAU to UAC;
- the stop codon UAA can be modified to UAG or UGA;
- the codon for Cys can be modified from UGU to UGC;
- the codon for His can be modified from CAU to CAC;
- the codon for Gln can be modified from CAA to CAG;
- the codons for Ile can be modified from AUU or AUA to AUC;
- the codons for Thr can be modified from ACU or ACA to ACC or ACG;
- the codon for Asn can be modified from AAU to AAC;
- the codon for Lys can be modified from AAA to AAG;
- the codons for Val can be modified from GUU or GUA to GUC or GUG;
- the codon for Asp can be modified from GAU to GAC;
- the codon for Glu can be modified from GAA to GAG.

[0063] In the case of the codons for Met (AUG) and Trp (UGG), on the other hand, there is no possibility of sequence modification.

[0064] The substitutions listed above can of course be used individually or also in all possible combinations to increase the G/C content of the antibody-coding RNA according to the invention compared with the native RNA sequence (and nucleic acid sequence, respectively). Thus, for example, all the codons for Thr occurring in the native RNA sequence can be modified to ACC (or ACG). Preferably, however, combinations of the above substitution possibilities are used, e.g.:

- substitution of all codons coding for Thr in the native RNA sequence by ACC (or ACG) and substitution of all codons

originally coding for Ser by UCC (or UCG or AGC);

- substitution of all codons coding for Ile in the native RNA sequence by AUC and substitution of all codons originally coding for Lys by AAG and substitution of all codons originally coding for Tyr by UAC;
- substitution of all codons coding for Val in the native RNA sequence by GUC (or GUG) and substitution of all codons originally coding for Glu by GAG and substitution of all codons originally coding for Ala by GCC (or GCG) and substitution of all codons originally coding for Arg by CGC (or CGG);
- substitution of all codons coding for Val in the native RNA sequence by GUC (or GUG) and substitution of all codons originally coding for Glu by GAG and substitution of all codons originally coding for Ala by GCC (or GCG) and substitution of all codons originally coding for Gly by GGC (or GGG) and substitution of all codons originally coding for Asn by AAC;
- substitution of all codons coding for Val in the native RNA sequence by GUC (or GUG) and substitution of all codons originally coding for Phe by UUC and substitution of all codons originally coding for Cys by UGC and substitution of all codons originally coding for Leu by CUG (or CUC) and substitution of all codons originally coding for Gln by CAG and substitution of all codons originally coding for Pro by CCC (or CCG);

etc.

[0065] Preferably, the G/C content of the coding region of the antibody-coding RNA according to the invention is increased compared with the G/C content of the coding region of the native RNA such that at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 % or more preferably at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or at least 55 %, even more preferably at least 60 %, at least 65 %, at least 70 % or at least 75 % and most preferably at least 80 %, at least 85 %, at least 90 %, at least 95 % or at least 100 % of the possible modifiable codons of the coding region of the native RNA (and nucleic acid, respectively) are modified.

[0066] In this connection, it is particularly preferable to increase to the maximum the G/C content of the antibody-coding RNA according to the invention, in particular in the coding region, compared with the native RNA sequence.

[0067] A second alternative of the antibody-coding RNA according to the invention with modifications is based on the knowledge that the translation efficiency of the RNA is also determined by a different frequency in the occurrence of tRNAs in cells. Thus, if so-called "rare" codons are present in an RNA sequence to an increased extent, the corresponding RNA is translated to a significantly poorer degree than in the case where codons which code for relatively "frequent" tRNAs are present.

[0068] According to this second alternative of the antibody-coding RNA according to the invention, the coding region of the RNA according to the invention is therefore modified compared with the coding region of the native RNA such that at least one codon of the native RNA which codes for a tRNA which is relatively rare in the cell is exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and which carries the same amino acid as the relatively rare tRNA.

[0069] By this modification, the sequence of the antibody-coding RNA according to the invention is modified such that codons for which frequently occurring tRNAs are available are inserted. Which tRNAs occur relatively frequently in the cell and which, in contrast, are relatively rare is known to a person skilled in the art; cf. e.g. Akashi, Curr. Opin. Genet. Dev. 2001, 11(6): 660-666.

[0070] According to the invention, by this modification all codons of the sequence of the antibody-coding RNA according to the invention which code for a tRNA which is relatively rare in the cell can be exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and which carries the same amino acid as the relatively rare tRNA.

[0071] It is particularly preferable to link the increased, in particular maximum, sequential G/C content in the antibody-coding RNA according to the invention with the "frequent" codons without modifying the amino acid sequence coded by the RNA according to the invention. This preferred embodiment provides a particularly efficiently translated and stabilized RNA sequence according to the invention which encodes an antibody (for example for a pharmaceutical composition according to the invention).

[0072] In the sequences of eukaryotic RNAs, there are typically destabilizing sequence elements (DSE) to which signal proteins bind and regulate the enzymatic degradation of the RNA *in vivo.* For further stabilization of the antibody-coding RNA according to the invention, one or more modifications compared with the corresponding region of the native RNA are therefore optionally carried out in the region coding for the protein, so that no destabilizing sequence elements are present. According to the invention, it is of course also preferable, where appropriate, to eliminate from the RNA DSEs present in the untranslated regions (3' and/or 5' UTR).

[0073] Such destabilizing sequences are, for example, AU-rich sequences ("AURES"), which occur in 3' UTR sections of numerous unstable RNAs (Caput et al., Proc. Natl. Acad. Sci. USA 1986, 83: 1670 to 1674). The antibody-coding RNA according to the invention is therefore preferably modified compared with the native RNA such that this no longer contains such destabilizing sequences. This also applies to those sequence motifs which are recognized by possible endonucleases, for example the sequence GAACAAG, which is contained in the 3' UTR segment of the gene which codes for the transferrin receptor (Binder et al., EMBO J. 1994, 13: 1969 to 1980). These sequence motifs are also

preferably eliminated in the antibody-coding RNA according to the invention.

[0074] A person skilled in the art is familiar with various methods which are suitable in the present case for substitution of codons in RNAs, i.e. substitution of codons in the antibody-coding RNA according to the invention. In the case of relatively short coding regions (which code for antibodies or antibody fragments as described here), for example, the total antibody-coding RNA according to the invention can be synthesized chemically using standard techniques such as are familiar to a person skilled in the art.

[0075] Nevertheless, base substitutions are preferably introduced using a DNA template for the preparation of the antibody-coding RNA according to the invention with the aid of techniques of the usual targeted mutagenesis (see, for example, Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd ed., Cold Spring Harbor, NY, 2001). In this method, for the preparation of the antibody-coding RNA according to the invention, a corresponding DNA molecule is therefore transcribed *in vitro* (see below). This DNA template optionally has a suitable promoter, for example a T3, T7 or SP6 promoter, for the *in vitro* transcription, which is followed by the desired nucleotide sequence for the antibody-coding RNA according to the invention to be prepared and a termination signal for the *in vitro* transcription. The DNA molecule which forms the template of the antibody-coding RNA construct to be prepared can be prepared by fermentative proliferation and subsequent isolation as part of a plasmid which can be replicated in bacteria. Plasmids which may be mentioned as suitable for this are, for example, the plasmids pT7Ts (GenBank accession number U26404; Lai et al., Development 1995, 121: 2349 to 2360), pGEM® series, for example pGEM®-1 (GenBank accession number X65300; from Promega) and pSP64 (GenBank accession number X65327); cf. also Mezei and Storts, Purification of PCR Products, in: Griffin and Griffin (ed.), PCR Technology: Current Innovation, CRC Press, Boca Raton, FL, 2001.

[0076] Using short synthetic RNA or DNA oligonucleotides which contain short single-stranded transitions at the cleavage sites formed, or genes prepared by chemical synthesis, the desired nucleotide sequence can thus be cloned into a suitable plasmid by molecular biology methods with which a person skilled in the art is familiar (cf. Maniatis *et al.,* (2001) supra). The RNA or DNA molecule is then cut out of the plasmid, in which it can be present in one or several copies, by digestion with restriction endonucleases.

[0077] According to a particular embodiment of the present invention, the antibody-coding (modified) RNA according to the invention described above, especially if the RNA is in the form of mRNA, can moreover have a 5' cap structure (a modified guanosine nucleotide). Examples of cap structures which may be mentioned, without being restricted thereto, are m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G.

[0078] According to a further preferred embodiment of the present invention, the antibody-coding (modified) RNA according to the invention contains, especially if the RNA is in the form of mRNA, a poly-A tail on the 3' terminus of typically about 10 to 200 adenosine nucleotides, preferably about 10 to 100 adenosine nucleotides, more preferably about 20 to 70 adenosine nucleotides or even more preferably about 20 to 60 adenosine nucleotides.

[0079] According to another preferred embodiment of the present invention, the antibody-coding (modified) RNA according to the invention contains, especially if the RNA is in the form of mRNA, a poly-C tail on the 3' terminus of typically about 10 to 200 cytosine nucleotides, preferably about 10 to 100 cytosine nucleotides, more preferably about 20 to 70 cytosine nucleotides or even more preferably about 20 to 60 or even 10 to 40 cytosine nucleotides. The poly-C tail may be added to the poly-A tail or may substitute the poly-A tail.

[0080] According to a further embodiment, the antibody-coding (modified) RNA according to the invention can additionally contain a nucleic acid section which codes a tag for purification. Such tags include, but without being limited thereto, e.g. a hexahistidine tag (His tag, polyhistidine tag), a streptavidin tag (Strep tag), an SBP tag (streptavidin-binding tag) a GST (glutathione S transferase) tag etc. The antibody-coding (modified) RNA according to the invention can furthermore encode a tag for purification via an antibody epitope (antibody-binding tag), e.g. a Myc tag, an Swa11 epitope, a FLAG tag, an HA tag etc., i.e. via recognition of the epitope via the (immobilized) antibody.

[0081] For efficient translation of RNA, in particular mRNA, effective binding of the ribosomes to the ribosome binding site (Kozak sequence: GCCGCCACCAUGG (SEQ ID NO: 16), the AUG forms the start codon) is necessary. In this respect, it has been found that an increased A/U content around this site renders possible a more efficient ribosome binding to the RNA. According to another preferred embodiment of the present invention, the antibody-coding (modified) RNA according to the invention can therefore have an increased A/U content around the ribosome binding site, preferably an A/U content which is increased by 5 to 50 %, more preferably one increased by 25 to 50 % or more, compared with the native RNA.

[0082] According to one embodiment of the antibody-coding (modified) RNA according to the invention, it is furthermore possible to insert one or more so-called IRES (internal ribosomal entry site) into the RNA. An IRES can thus function as the sole ribosome binding site, but it can also serve to provide an antibody-coding (modified) RNA according to the invention which codes for several antibodies or antibody fragments or for at least one antibody or antibody fragment which are to be translated by the ribosomes independently of one another ("multicistronic RNA"). Such an RNA can code, for example, a complete sequence of an antibody, the corresponding coding regions of the heavy and light chain being linked (functionally) with one another by an IRES sequence. However, the heavy and light chain to be encoded by the inventive RNA may also be located in one single "cistron". According to the invention, the IRES sequences

described are employed in particular for (virtually) simultaneous and uniform expression of the light and the heavy chains of the antibody coded by the RNA according to the invention. Examples of IRES sequences which can be used according to the invention are those from picornaviruses (e.g. FMDV), pestiviruses (CFFV), polioviruses (PV), encephalomyocarditis viruses (ECMV), foot and mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukoma virus (MLV), simian immunodeficiency viruses (SIV), cricket paralysis viruses (CrPV) or an SIRES sequence.

[0083] According to a further preferred embodiment of the present invention, the antibody-coding (modified) RNA according to the invention has, in the 5' and/or 3' untranslated regions, stabilizing sequences which are capable of increasing the half-life of the RNA in the cytosol. These stabilizing sequences can have a 100 % sequence homology to naturally occurring sequences which occur in viruses, bacteria and eukaryotes, but can also be partly or completely synthetic in nature. The untranslated sequences (UTR) of the β-globin gene, for example from *Homo sapiens* or *Xenopus laevis,* may be mentioned as an example of stabilizing sequences which can be used in the present invention. Another example of a stabilizing sequence has the general formula (C/U)CCAN$_x$CCC(U/A)Py$_x$UC(C/U)CC (SEQ ID NO: 17), which is contained in the 3' UTR of the very stable RNA which codes for α-globin, α-(I)-collagen, 15-lipoxygenase or for tyrosine hydroxylase (cf. Holcik et al., Proc. Natl. Acad. Sci. USA 1997, 94: 2410 to 2414). Such stabilizing sequences can of course be used individually or in combination with one another and also in combination with other stabilizing sequences known to a person skilled in the art.

[0084] In a further preferred embodiment, the antibody-coding (modified) RNA according to the invention can encode a secretory signal peptide, in addition to the antibodies as described here. Such signal peptides are (signal) sequences which conventionally comprise a length of from 15 to 30 amino acids and are preferably localized on the N-terminus of the coded antibody. Signal peptides typically render possible transport of a protein or peptide fused therewith (here e.g. an antibody) to or into a defined cell compartment, preferably the cell surface, the endoplasmic reticulum or the endosomal-lysosomal compartment. Examples of signal sequences which can be used according to the invention are e.g. signal sequences of conventional and non-conventional MHC molecules, cytokines, immunoglobulins, the invariant chain, Lamp1, tapasin, Erp57, calreticulin and calnexin, and all further membrane-located, endosomally-lysosomally or endoplasmic reticulum-associated proteins. The signal peptide of the human MHC class I molecule HLA-A*0201 is preferably used.

[0085] Sequences which render possible transport of a protein or peptide fused therewith (here e.g. an antibody) to or into a defined cell compartment, preferably the cell surface, the nucleus, the nucleus region, the plasma membrane, the cytosol, the endoplasmic reticulum, the organelles, the mitochondria, the Golgi apparatus or the endosomal-lysosomal compartment, also include, without being limited thereto, so-called routing signals, sorting signals, retention signals or salvage signals and membrane topology-stop transfer signals (cf. Pugsley, A. P., Protein Targeting, Academic Press, Inc. (1989)) at the level of the RNA according to the invention. In this connection, localization sequences include nucleic acid sequences which encode e.g. signals, i.e. amino acid sequences, such as, for example, KDEL (SEQ ID NO: 18) (Munro, et al., Cell 48:899-907 (1987)) DDEL (SEQ ID NO: 19), DEEL (SEQ ID NO: 20), QEDL (SEQ ID NO: 21) and RDEL (SEQ ID NO: 22) (Hangejorden, et al., J. Biol. Chem. 266:6015 (1991)) for the endoplasmic reticulum; PKKKRKV (SEQ ID NO: 23) (Lanford, et al. Cell 46:575 (1986)) PQKKIKS (SEQ ID NO: 24) (Stanton, L. W., et al., Proc. Natl. Acad. Sci USA 83:1772 (1986); QPKKP (SEQ ID NO: 25) (Harlow, et al., Mol. Cell Biol. 5:1605 1985), and RKKR (SEQ ID NO:26) for the nucleus; and RKKRRQRRRAHQ (SEQ ID NO: 27) (Seomi, et al., J. Virology 64:1803 (1990)), RQARRNRRRRWRERQR (SEQ ID NO: 28) (Kubota, et al., Biochem. and Biophy, Res. Comm. 162:963 (1989)), and MPLTRRRPAASQALAPPTP (SEQ ID NO: 29) (Siomi, et al., Cell 55:197 (1988)) for the nucleus region; MDDQRDLISNNEQLP (SEQ ID NO: 30) (Bakke, et al., Cell 63:707-716 (1990)) for the endosomal compartment (see, for example, Letoumeur, et al., Cell 69:1183 (1992) for the targeting of liposomes). Myristoylation sequences can furthermore be used in order to lead the expressed protein or peptide (here e.g. an antibody) to the plasma membrane, or to certain various sub-cell compartments, such as the nucleus region, the organelles, the mitochondria and the Golgi apparatus. Corresponding amino acid sequences which are coded by a corresponding codon sequence of the RNA according to the invention are given below. The sequence MLFNLRXXLNNAAFRHGHNFMVRNFRCGQPLX (SEQ ID NO: 31) can be used to lead the antibody to the mitochondrial matrix (Pugsley, supra). See Tang, et al., J. Bio. Chem. 207:10122, in respect of the localization of proteins (antibodies) to the Golgi apparatus; for the localization of proteins to the plasma membrane: GCVCSSNP (SEQ ID NO: 32), GQTVTTPL (SEQ ID NO: 33), GQELSQHE (SEQ ID NO: 34), GNSPSYNP (SEQ ID NO: 35), GVSGSKGQ (SEQ ID NO: 36), GQTITTPL (SEQ ID NO: 37), GQTLTTPL (SEQ ID NO: 38), GQIFSRSA (SEQ ID NO: 39), GQIHGLSP (SEQ ID NO: 40), GARASVLS (SEQ ID NO: 41), and GCTLSAEE (SEQ ID NO: 42); to the endoplasmic reticulum GQNLSTSN (SEQ ID NO: 43); to the nucleus GAALTILV (SEQ ID NO: 44) and GAALTLLG (SEQ ID NO: 45); to the endoplasmic reticulum and to the cytoplasm GAQVSSQK (SEQ ID NO: 46) and GAQLSRNT (SEQ ID NO: 47); to the Golgi apparatus, to the nucleus, to the cytoplasm and to the cytoskeleton: GNAAAAKK (SEQ ID NO: 48); to the cytoplasm and to the cytoskeleton GNEASYPL (SEQ ID NO: 49); and to the plasma membrane and to the cytoskeleton GSSKSKPK (SEQ ID NO: 50). Such sequences as described above are preferably used for RNAs which code for intrabodies, i.e antibodies which are retained in the cell and are not secreted.

[0086] The modifications described here can be introduced into the antibody-coding RNA sequence according to the invention in a suitable manner by a person skilled in the art. For example, the optimum modified RNA according to the invention can be determined by methods known to the person skilled in the art, e.g. the G/C content can be adapted manually and/or by means of an automated method as disclosed in WO 02/098443. In this context, the RNA sequences can be adapted with the various additional optimization aims described here: On the one hand, the adaptation can be carried out with the highest possible G/C content, and on the other hand taking into the best possible account the frequency of the tRNAs according to codon usage. In this context, in the first step of the method a virtual translation of any desired RNA (or DNA) sequence is carried out in order to generate the corresponding amino acid sequence. Starting from the amino acid sequence, a virtual reverse translation is carried out, which on the basis of the degenerated genetic code provides selection possibilities for the corresponding codons. Depending on the optimization or modification required, corresponding selection lists and optimization algorithms are used for selection of the suitable codons. The algorithm is typically implemented on a computer with the aid of suitable software. The optimized RNA sequence is established in this way and can be displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. The same also applies to the frequency of the individual nucleotides. In this context, the changes compared with the original nucleotide sequence are preferably highlighted. According to a preferred embodiment, stable sequences which are known in nature and can provide the basis for an RNA stabilized in accordance with natural sequence motifs are furthermore read in. A secondary structure analysis which can analyse stabilizing and destabilizing properties or, respectively, regions of the RNA with the aid of structure calculations can likewise be envisaged.

[0087] Furthermore, according to a preferred embodiment effective transfer of the antibody-coding (modified) RNA according to the invention into the cells to be treated or the organism to be treated can be improved by complexing the antibody-coding (modified) RNA according to the invention with a cationic peptide or protein or binding it thereto. Such a complexing/condensing of the RNA, in particular mRNA, includes, for example, complexing (or binding) of the RNA according to the invention with a (poly)cationic poly-mer, polyplexes, protein(s), in particular polycationic protein(s), or peptide(s). Preferably, an RNA (mRNA) according to the invention is complexed or condensed with at least one cationic or polycationic agent. Preferably, such a cationic or polycationic agent is an agent which is chosen from the group consisting of protamine, poly-L-lysine, poly-L-arginine, nucleolin, spermin and histones, nucleolin or derivatives thereof. The use of protamine as a polycationic, nucleic acid-binding protein is particularly preferred. This procedure for stabilizing RNA is described, for example, in EP-A-1083232, the disclosure content of which in this respect is included in its full scope in the present invention.

[0088] According to a particular embodiment, the antibody-coding (modified) RNA according to the invention can contain a lipid modification. Such an RNA modified with a lipid typically comprises an antibody-coding RNA, as defined here, according to the invention, at least one linker covalently linked with this RNA and at least one lipid covalently linked with the particular linker. Alternatively, the (modified) RNA according to the invention modified with a lipid comprises (at least) one (modified) RNA, as defined here, according to the invention and at least one (bifunctional) lipid covalently linked with this RNA. According to a third alternative the (modified) RNA according to the invention modified with a lipid comprises a (modified) RNA, as defined here, according to the invention, at least one linker covalently linked with this RNA and at least one lipid linked covalently with the particular linker and at least one (bifunctional) lipid covalently linked (without a linker) with this (modified) RNA according to the invention.

[0089] The lipid employed for lipid modification of the antibody-coding (modified) RNA according to the invention is typically a lipid or a lipophilic residue, which is preferably biologically active *per se.* Such lipids preferably include natural substances, or compounds, such as e.g. vitamins, e.g. α-tocopherol (vitamin E), including RRR-α-tocopherol (formerly D-α-tocopherol), L-α-tocopherol, the racemate D,L-α-tocopherol, vitamin E succinate (VES) or vitamin A and derivatives thereof, e.g. retinic acid, retinol, vitamin D and derivatives thereof, e.g. vitamin D and ergosterol precursors thereof, vitamin E and derivatives thereof, vitamin K and derivatives thereof, e.g. vitamin K and related quinone or phytol compounds, or steroids, such as bile acids, for example cholic acid, deoxycholic acid, dehydrocholic acid, cortisone, digoxygenin, testosterone, cholesterol or thiocholesterol. Further lipids or lipophilic residues in the context of the present invention include, without being limited thereto, polyalkylene glycols, (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533), aliphatic groups, such as e.g. C_1-C_{20}-alkanes, C_1-C_{20}-alkenes, or C_1-C_{20}-alkanol compounds etc., such as, for example, dodecanediol, hexadecanol or undecyl residues (Saison-Behmoaras et al., EMBO J, 1991, 10, 111; Kabanov et al., FEBS Lett., 1990, 259, 327; Svinarchuk et al., Biochimie, 1993, 75, 49), phospholipids, such as e.g. phosphatidylglycerol, diacylphosphatidylglycerol, phosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, dihexadecyl-rac-glycerol, sphingolipids, cerebrosides, gangliosides, or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651; Shea et al., Nucl. Acids Res., 1990, 18, 3777), polyamines or polyalkylene glycols, such as e.g. polyethylene glycol (PEG) (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969), hexaethylene glycol (HEG), palmitin, or palmityl residues (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229), octadecylamines, or hexylaminocarbonyloxycholesterol residues (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923), and waxes, terpenes, alicyclic hy-

drocarbons, saturated or mono- or polyunsaturated fatty acid residues etc.

[0090] The linking between the lipid and the antibody-coding (modified) RNA according to the invention can in principle take place on any nucleotide, on the base or the sugar residue of any nucleotide, on the 3' and/or 5' end, and/or on the phosphate backbone of the antibody-coding (modified) RNA according to the invention. According to the invention, a terminal lipid modification of the (modified) RNA according to the invention on the 3' and/or 5' end thereof is particularly preferred. A terminal modification has several advantages over modifications within the sequence. On the one hand, modifications within the sequence can influence the hybridization properties, which may have an adverse effect in the case of sterically demanding residues. (Sterically demanding) modifications within the sequence very often also interfere in translation, which can frequently lead to an interruption in the protein synthesis. On the other hand, in the case of preparation by synthesis of a lipid-modified (modified) RNA according to the invention which is modified exclusively terminally, synthesis of this antibody-coding (modified) RNA according to the invention is carried out with monomers which are commercially available in large amounts, and synthesis protocols known in the prior art are used.

[0091] According to a first preferred embodiment, the linking takes place between the antibody-coding (modified) RNA according to the invention and at least one lipid via a linker (linked covalently with the (modified) RNA). Linkers in the context of the present invention typically contain at least two and optionally 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30 or more reactive groups, chosen from e.g. a hydroxyl group, an amino group, an alkoxy group etc. One reactive group preferably serves to bond the antibody-coding (modified) RNA according to the invention described here. This reactive group can be in a protected form, e.g. as a DMT (dimethoxytrityl chloride) group, as an Fmoc group, as an MMT (monomethoxytrityl) group, as a TFA (trifluoroacetic acid) group etc. Sulfur groups can furthermore be protected by disulfides, e.g. alkylthiols, such as, for example, 3-thiopropanol, or with activated components, such as 2-thiopyridine. According to the invention, one or more further reactive groups serve for covalent bonding of one or more lipids. According to the first embodiment, an antibody-coding (modified) RNA according to the invention can therefore bond at least one lipid via the covalently bonded linker, e.g. 1, 2, 3, 4, 5, 5-10, 10-20, 20-30 or more lipid(s), particularly preferably at least 3-8 or more lipids per (modified) RNA. In this context, the bonded lipids can be bonded separately from one another at various positions of the antibody-coding (modified) RNA according to the invention, but can also be in the form of a complex at one or more positions of the (modified) RNA. An additional reactive group of the linker can be used for direct or indirect (cleavable) bonding to a carrier material, e.g. a solid phase. Preferred linkers according to the present invention are e.g. glycol, glycerol and glycerol derivatives, 2-aminobutyl-1,3-propanediol and 2-aminobutyl-1,3-propanediol derivatives/matrix, pyrrolidine linkers or pyrrolidine-containing organic molecules (in particular for a modification on the 3' end) etc. According to the invention, glycerol or glycerol derivatives (C_3 anchor) or a 2-aminobutyl-1,3-propanediol derivative/matrix (C_7 anchor) are particularly preferably used as linkers. A glycerol derivative (C_3 anchor) as a linker is particularly preferred if the lipid modification can be introduced via an ether bond. If the lipid modification is to be introduced e.g. via an amide or an urethane bond, e.g. a 2-aminobutyl-1,3-propanediol matrix (C_7 anchor) is preferred. In this connection, the bond formed between the linker and the antibody-coding (modified) RNA according to the invention is preferably such that it is compatible with the conditions and chemicals of amidite chemistry, that is to say it is preferably neither acid- nor base-labile. In particular, those bonds which are readily accessible synthetically and are not hydrolysed by the ammoniacal cleavage procedure of a nucleic acid synthesis process are preferred. Possible bonds are in principle all appropriately suitable bonds, preferably ester bonds, amide bonds, urethane bonds and ether bonds. In addition to the good accessibility of the educts (few synthesis stages), the ether bond is particularly preferred in this context because of its relatively high biological stability to enzymatic hydrolysis.

[0092] According to a second preferred embodiment, for the lipid modification of the (modified) RNA according to the invention the linking of (at least one) (modified) RNA according to the invention takes place directly with at least one (bifunctional) lipid as described here, i.e. without using a linker as described here. In this case, the (bifunctional) lipid according to the invention preferably contains at least two reactive groups, or optionally 3, 4, 5, 6, 7, 8, 9, 10 or more reactive groups, a first reactive group serving for direct or indirect bonding of the lipid to a carrier material described here and at least one further reactive group serving for bonding of the (modified) RNA. According to the second embodiment, an antibody-coding (modified) RNA according to the invention can therefore preferably bond at least one lipid (directly without a linker), e.g. 1, 2, 3, 4, 5, 5-10, 10-20, 20-30 or more lipid(s), particularly preferably at least 3-8 or more lipids per (modified) RNA. In this context, the bonded lipids can be bonded separately from one another at various positions of the antibody-coding (modified) RNA according to the invention, but can also be in the form of a complex at one or more positions of the (modified) RNA. Alternatively, according to the second embodiment, at least one antibody-coding (modified) RNA, e.g. optionally 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30 or more (modified) RNAs, according to the invention can be bonded to a lipid as described above via reactive groups thereof. Lipids which can be used for this second embodiment particularly preferably include such (bifunctional) lipids which render possible a coupling (preferably on their termini or optionally intramolecularly), such as e.g. polyethylene glycol (PEG) and derivatives thereof, hexaethylene glycol (HEG) and derivatives thereof, alkanediols, aminoalkanes, thioalkanols etc. The bond between a (bifunctional) lipid and an antibody-coding (modified) RNA according to the invention as described above is preferably such as is described for the first preferred embodiment.

[0093] According to a third embodiment, for the lipid modification of the (modified) RNA according to the invention the linking between the antibody-coding (modified) RNA according to the invention and at least one lipid as described here takes place via both of the abovementioned embodiments simultaneously. Thus e.g. the antibody-coding (modified) RNA according to the invention can be linked at one position of the RNA with at least one lipid via a linker (analogously to the 1st embodiment) and at another position of the (modified) RNA directly with at least one lipid without using a linker (analogously to the 2nd embodiment). For example, at least one lipid as described here can be linked covalently with the RNA at the 3' end of the (modified) RNA via a linker, and a lipid as described here can be linked covalently with the RNA at the 5' end of the (modified) RNA without a linker. Alternatively, at least one lipid as described here can be linked covalently with the (modified) RNA at the 5' end of an antibody-coding (modified) RNA according to the invention via a linker, and a lipid as described here can be linked covalently with the (modified) RNA at the 3' end of the (modified) RNA without a linker. Covalent linkings can likewise take place not only on the termini of the antibody-coding (modified) RNA according to the invention, but also intramolecularly, as described above, e.g. on the 3' end and intramolecularly, on the 5' end and intramolecularly, on the 3' and 5' end and intramolecularly, exclusively intramolecularly etc.

[0094] The (modified) RNA according to the invention described here can be prepared by preparation processes known in the prior art, e.g. automatically or manually via known nucleic acid syntheses (see, for example, Maniatis *et al.* (2001) supra) or also via molecular biology methods, for example with subsequent purification, for example via chromatography methods.

[0095] According to further subject matter of the present invention, the antibody-coding (modified) RNA according to the invention can be used for the preparation of a pharmaceutical composition for treatment of tumours and cancer diseases, cardiovascular diseases, infectious diseases, autoimmune diseases or optionally monogenetic diseases, e.g. in gene therapy.

[0096] A pharmaceutical composition in the context of the present invention comprises an antibody-coding (modified) RNA as described here and optionally a pharmaceutically suitable carrier and/or further auxiliary substances and additives. The pharmaceutical composition employed according to the present invention typically comprises a safe and effective amount of a (modified) RNA as described here. As used here, "safe and effective amount" means an amount of the antibody-coding (modified) RNA according to the invention such as is sufficient to induce significantly, by expression of the coded antibody, a positive change of a state to be treated, e.g. a tumour disease or cancer disease, a cardiovascular disease or an infectious disease, as described in the following. At the same time, however, a "safe and effective amount" is low enough to avoid serious side effects in the therapy of these diseases, that is to say to render possible a reasonable ratio of advantage and risk. Determination of these limits typically lies within the range of reasonable medical judgement. The concentration of the antibody-coding (modified) RNA according to the invention in such pharmaceutical compositions can therefore vary, for example, without being limited thereto, within a wide range of from e.g. 0.1 ng to 1,000 mg/ml. Such a "safe and effective amount" of an antibody-coding (modified) RNA according to the invention can vary in connection with the particular state to be treated and the age and the physical state of the patient to be treated, the severity of the state, the duration of the treatment, the nature of the concomitant therapy, of the particular pharmaceutically suitable carrier used and similar factors within the knowledge and experience of the treating doctor. The pharmaceutical composition described here can be employed for human and also for veterinary medicine purposes.

[0097] The pharmaceutical composition according to the invention described here can optionally comprise a pharmaceutically suitable carrier (and/or vehicle). The term "pharmaceutically suitable carrier (and/or vehicle)" used here preferably includes one or more compatible solid or liquid carriers or vehicles, (e.g. fillers, or diluents or encapsulating compounds) which are suitable for administration to a person. The term "compatible" as used here means that the constituents of the composition are capable of being mixed together with the antibody-coding (modified) RNA according to the invention and the auxiliary substance optionally contained in the composition, as such and with one another in a manner such that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the composition under usual condition of use, such as e.g. would reduce the pharmaceutical activity of the coded antibody or even suppress or impair expression of the coded antibody. Pharmaceutically suitable carrier must of course have a sufficiently high purity and a sufficiently low toxicity to render them suitable for administration to a person to be treated.

[0098] Pharmaceutically suitable carriers or vehicles, that may be used in the inventive pharmaceutical composition, may be typically distinguished into solid or liquid carriers or vehicles, wherein a specific determination may depend on the viscosity of the respective carrier or vehicle to be used.

[0099] In this context, solid carriers and vehicles typically include e.g., but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, and salts, if provided in solid form, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, or polyvinyl pyrrolidone, waxes, polyethylene-polyoxypropylene-block polymers, wool fat, sugars, such as, for example, lactose, glucose and sucrose; starches, such as, for example, corn starch or potato starch; or cellulose-based substances, e.g. cellulose and its derivatives, such as, for example, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; pulverized tragacanth; malt; gelatine; tallow; solid lubricants, such as, for example, stearic acid, magnesium stearate; calcium sulfate; wetting agents, such as, for example, sodium lauryl sulfate; colouring agents; flavouring agents; drug (active agent)

carriers; tablet-forming agents; stabilizers; antioxidants; preservatives; etc..

[0100] Liquid carriers or vehicles, e.g. for aqueous or oleaginous suspensions, typically include, but are not limited to, e.g., water; pyrogen-free water; solutions of ion exchangers, alumina, aluminum stearate, lecithin, or serum proteins, such as human serum albumin; alginic acid; isotonic saline solutions or phosphate-buffered solutions, Ringer's solution, isotonic sodium chloride solution, etc. or salts or electrolytes, if provided in solubilized form, such as protamine sulfate, phosphates, e.g. disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, or (other) buffer substances including e.g. glycine, sorbic acid, potassium sorbate; liquid solutions of polyols, such as, for example, polyethylene glycol, polypropylene glycol, glycerol, 1,3-butanediol, sorbitol, Mannitol; sterile, fixed oils, any suitable bland fixed oil, e.g. including synthetic mono- or di-glycerides, partial glyceride mixtures of saturated vegetable fatty acids, fatty acids, such as oleic acid and its glyceride derivatives, natural pharmaceutically-acceptable oils, e.g. plant oils, such as, for example, groundnut oil, cottonseed oil, sesame oil, corn oil and oil from Theobroma; olive oil or castor oil, especially in their polyoxyethylated versions. These liquid carriers or vehicles may also contain or comprise a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents, or commonly used surfactants or emulsifiers, such as Tween®, Spans and other emulsifying agents or bioavailability enhancers, etc., if provided in a liquid form.

[0101] The choice of a pharmaceutically suitable carrier as described above is determined in particular by the mode in which the pharmaceutical composition according to the invention is administered. The pharmaceutical composition according to the invention can be administered, for example, systemically. Administration routes include e.g. transdermal, oral, parenteral, including subcutaneous or intravenous injections, topical and/or intranasal routes. The suitable amount of the pharmaceutical composition according to the invention which is to be used can be determined by routine experiments using animal models. Such models include, but without being limited thereto, models of the rabbit, sheep, mouse, rat, dog and non-human primate models. Preferred unit dose forms for injection include sterile solutions of water, physiological saline solution or mixtures thereof. The pH of such solutions should be adjusted to about 7.4. Suitable carriers for injection include hydrogels, devices for controlled or delayed release, polylactic acid and collagen matrices. Pharmaceutically suitable carriers which can be used here include those which are suitable for use in lotions, creams, gels and the like. If the compound is to be administered perorally, tablets, capsules and the like are the preferred unit dose form. The pharmaceutically suitable carriers for the preparation of unit dose forms which can be used for oral administration are well-known in the prior art. Their choice will depend on secondary considerations, such as flavour, cost and storage stability, which are not critical for the purposes of the present invention and can be implemented without difficulties by a person skilled in the art.

[0102] The pharmaceutical composition according to the invention can furthermore comprise an injection buffer, which preferably improves the transfection and also the translation of the antibody-coding RNA according to the invention in cells or an organism. The pharmaceutical composition according to the invention can comprise, for example, an aqueous injection buffer which contains, with respect to the total pharmaceutical composition, if this is in liquid form, a sodium salt, preferably at least 50 mM sodium salt, a calcium salt, preferably at least 0.01 mM calcium salt, and optionally a potassium salt, preferably at least 3 mM potassium salt. According to a preferred embodiment, the sodium salts, calcium salts and optionally potassium salts contained in such an injection buffer are in the form of halides, e.g. chlorides, iodides or bromides, or in the form of their hydroxides, carbonates, bicarbonates or sulfates. Examples which are to be mentioned here are, for the sodium salt NaCl, NaI, NaBr, Na_2CO_3, $NaHCO_3$, Na_2SO_4, for the potassium salt optionally present KCl, KI, KBr, K_2CO_3, $KHCO_3$, K_2SO_4, and for the calcium salt $CaCl_2$, CaI_2, $CaBr_2$, $CaCO_3$, $CaSO_4$, $Ca(OH)_2$. The injection buffer can also contain organic anions of the abovementioned cations. In a particularly preferred embodiment, such an injection buffer contains as salts sodium chloride (NaCl), calcium chloride ($CaCl_2$) and optionally potassium chloride (KCl), it also being possible for other anions to be present in addition to the chlorides.

[0103] These salts are typically present in the injection buffer optionally used in the pharmaceutical composition according to the invention, with respect to the total pharmaceutical composition (if this is in liquid form), in a concentration of at least 50 mM sodium chloride (NaCl), at least 3 mM potassium chloride (KCl) and at least 0.01 mM calcium chloride ($CaCl_2$). The injection buffer can be in the form of both hypertonic and isotonic or hypotonic injection buffers. In connection with the present invention, in this context the injection buffer is hypertonic, isotonic or hypotonic with respect to the particular reference medium, i.e. the injection buffer has either a higher, the same or a lower salt content compared with the particular reference medium, such concentrations of the abovementioned salts which do not lead to damage to the cells caused by osmosis or other concentration effects preferably being employed. Reference media here are, for example, liquids which occur in "in vivo" methods, such as, for example, blood, lymph fluid, cytosol fluids or other fluids which occur in the body, or liquids or buffers conventionally employed in "in vitro" methods. Such liquids and buffers are known to a person skilled in the art.

[0104] The injection buffer optionally contained in the pharmaceutical composition according to the invention can also contain further components, for example sugars (mono-, di-, tri- or polysaccharides), in particular glucose or mannitol. In a preferred embodiment, however, no sugars are present in the injection buffer used. It is also preferable for the injection buffer precisely to contain no non-charged components, such as, for example, sugars. The injection buffer

typically contains exclusively metal cations, in particular from the group consisting of the alkali or alkaline earth metals, and anions, in particular the anions described above. The pH of the injection buffer used, with respect to the total pharmaceutical composition, if this is in liquid form, is preferably between 1 and 8.5, preferably between 3 and 5, more preferably between 5.5 and 7.5, in particular between 5.5 and 6.5. If appropriate, the injection buffer can also contain a buffer system which fixes the injection buffer at a buffered pH. This can be, for example, a phosphate buffer system, HEPES or Na_2HPO_4/NaH_2PO_4 However, the injection buffer used very particularly preferably contains none of the abovementioned buffer systems or contains no buffer system at all.

[0105] The injection buffer optionally contained in the pharmaceutical composition according to the invention can contain, in addition to or as an alternative to the monovalent and divalent cations described, divalent cations, in particular from the group consisting of alkaline earth metals, such as, for example, magnesium (Mg^{2+}), or also iron (Fe^{2+}), and monovalent cations, in particular from the groups consisting of alkali metals, such as, for example, lithium (Li^+). These monovalent cations are preferably in the form of their salts, e.g. in the form of halides, e.g. chlorides, iodides or bromides, or in the form of their hydroxides, carbonates, bicarbonates or sulfates. Examples which are to be mentioned here are, for the lithium salt LiCl, LiI, LiBr, Li_2CO_3, $LiHCO_3$, Li_2SO_4, for the magnesium salt $MgCl_2$, MgI_2, $MgBr_2$, $MgCO_3$, $MgSO_4$, and $Mg(OH)_2$, and for the iron salt $FeCl_2$, $FeBr_2$, FeI_2, FeF_2, Fe_2O_3, $FeCO_3$, $FeSO_4$, $Fe(OH)_2$. All the combinations of di- and/or monovalent cations, as described above, are likewise included. Such injection buffers which contain only divalent, only monovalent or di- and monovalent cations can thus be used in the pharmaceutical composition according to the invention. Such injection buffers which contain only one type of di- or monovalent cations, particularly preferably e.g. only Ca^{2+} cations, or a salt thereof, e.g. $CaCl_2$, can likewise be used. The molarities given above for Ca^{2+} (as a divalent cation) and Na^{1+} (as a monovalent cation) (that is to say typically concentrations of at least 50 mM Na^+, at least 0.01 mM Ca^{2+} and optionally at least 3 mM K^+) in the injection buffer can also be taken into consideration if another di- or monovalent cation, in particular other cations from the group consisting of the alkaline earth metals and alkali metals, are employed instead of some or all the Ca^{2+} or, respectively, Na^{1+} in the injection buffer used according to the invention for the preparation of the injection solution. All the Ca^{2+} or Na^{1+}, as mentioned above, can indeed be replaced by other di- or, respectively, monovalent cations in the injection buffer used, for example also by a combination of other divalent cations (instead of Ca^{2+}) and/or a combination of other monovalent cations (instead of Na^{1+}) (in particular a combination of other divalent cations from the group consisting of the alkaline earth metals or, respectively, of other monovalent cations from the group consisting of the alkali metals), but it is preferable to replace at most some of the Ca^{2+} or Na^{1+}, i.e. for at least 20 %, preferably at least 40 %, even more preferably at least 60 % and still more preferably at least 80 % of the particular total molarities of the mono- and divalent cations in the injection buffer to be occupied by Ca^{2+} and, respectively, Na^{1+}. However, it is very particularly preferable if the injection buffer optionally contained in the pharmaceutical composition according to the invention contains exclusively Ca^{2+} as a divalent cation and Na^{1+} as a monovalent cation, that is to say, with respect to the total pharmaceutical composition, Ca^{2+} represents 100 % of the total molarity of divalent cations, just as Na^{1+} represents 100 % of the total molarity of monovalent cations. The aqueous solution of the injection buffer can contain, with respect to the total pharmaceutical composition, up to 30 mol% of the salts contained in the solution, preferably up to 25 mol%, preferably up to 20 mol%, furthermore preferably up to 15 mol%, more preferably up to 10 mol%, even more preferably up to 5 mol%, likewise more preferably up to 2 mol% of insoluble or sparingly soluble salts. Salts which are sparingly soluble in the context of the present invention are those of which the solubility product is $< 10^{-4}$. Salts which are readily soluble are those of which the solubility product is $> 10^{-4}$. Preferably, the injection buffer optionally contained in the pharmaceutical composition according to the invention is from 50 mM to 800 mM, preferably from 60 mM to 500 mM, more preferably from 70 mM to 250 mM, particularly preferably 60 mM to 110 mM in sodium chloride (NaCl), from 0.01 mM to 100 mM, preferably from 0.5 mM to 80 mM, more preferably from 1.5 mM to 40 mM in calcium chloride ($CaCl_2$) and optionally from 3 mM to 500 mM, preferably from 4 mM to 300 mM, more preferably from 5 mM to 200 mM in potassium chloride (KCl). Organic anions can also occur as further anions in addition to the abovementioned inorganic anions, for example halides, sulfates or carbonates. Among these there may be mentioned succinate, lactobionate, lactate, malate, maleate etc., which can also be present in combination.

[0106] An injection buffer optionally contained in the pharmaceutical composition according to the invention preferably contains lactate. If it contains an organic anion, such an injection buffer particularly preferably contains exclusively lactate as the organic anion. Lactate in the context of the invention can be any desired lactate, for example L-lactate and D-lactate. Lactate salts which occur in connection with the present invention are typically sodium lactate and/or calcium lactate, especially if the injection buffer contains only Na^+ as a monovalent cation and Ca^{2+} as a divalent cation. An injection buffer optionally used in the pharmaceutical composition according to the invention and as described above preferably contains, with respect to the total pharmaceutical composition, from 15 mM to 500 mM, more preferably from 15 mM to 200 mM, and even more preferably from 15 mM to 100 mM lactate. In this context, it has been found that the use of an injection buffer with the components described above, optionally with or without lactate (in the following: "RL injection buffer" if it does not contain the component lactate, or "RL injection buffer with lactate" if it does contain the component lactate) for RNA injection solutions (i.e. injection solutions which contain RNA and are suitable for injection of this RNA) significantly increases both the transfer and the translation of the RNA into/in the cells/tissue of a host

organism (mammal) compared with other injection buffers conventionally used in the prior art.

[0107] According to a particular embodiment, the pharmaceutical composition used here can also be provided as a passive vaccine (for passive immunization). In the present invention, without being restricted to a theory, passive immunization is based on the introduction of an antibody-coding (modified) RNA as described here into an organism, in particular into a cell, the coded antibody then being expressed, i.e. translated. As a result, binding of such molecules, e.g. nucleic acids or antigens, for which the coded antibody is specific can take place. Passive vaccines in connection with the present invention typically comprise a composition as described above for a pharmaceutical composition, the composition of such passive vaccines used being determined in particular by the mode in which they are administered. Preferably, the passive vaccines according to the invention are administered systemically or in some cases non-systemically. Administration routes of such passive vaccines according to the invention typically include transdermal, oral, parenteral, including subcutaneous, intravenous, or intraarterial injections, topical and/or intranasal routes. Passive vaccines according to the invention are therefore preferably formulated in a liquid or solid form.

[0108] According to further preferred subject matter of the present invention, the antibody-coding (modified) RNA according to the invention or a pharmaceutical composition according to the invention is used for treatment of indications mentioned by way of example in the following. Without being limited thereto, diseases or states, for example, such as e.g. cancer or tumour diseases chosen from melanomas, malignant melanomas, colon carcinomas, lymphomas, sarcomas, blastomas, kidney carcinomas, gastrointestinal tumours, gliomas, prostate tumours, bladder cancer, rectal tumours, stomach cancer, oesophageal cancer, pancreatic cancer, liver cancer, mammary carcinomas (= breast cancer), uterine cancer, cervical cancer, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), hepatomas, diverse virus-induced tumours, such as e.g. papilloma virus-induced carcinomas (e.g. cervix carcinoma = cervical cancer), adenocarcinomas, herpes virus-induced tumours (e.g. Burkitt's lymphoma, EBV-induced B cell lymphoma), hepatitis B-induced tumours (hepatocell carcinomas), HTLV-1- and HTLV-2-induced lymphomas, acusticus neurinoma, lung carcinomas (= lung cancer = bronchial carcinoma), small cell lung carcinomas, throat cancer, anal carcinoma, glioblastoma, rectum carcinoma, astrocytoma, brain tumours, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, testicular cancer, thyroid carcinoma, Hodgkin's syndrome, meningeomas, Schneeberger's disease, pituitary tumour, mycosis fungoides, carcinoids, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, kidney cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumours, oligodendroglioma, vulval cancer, intestinal cancer, colon carcinoma, oesophageal carcinoma (= oesophageal cancer), wart conditions, small intestine tumours, craniopharyngeomas, ovarian carcinoma, soft tissue tumours (sarcomas), ovarian cancer (= ovarian carcinoma), pancreatic carcinoma (= pancreatic cancer), endometrium carcinoma, liver metastases, penis cancer, tongue cancer, gallbladder cancer, leukaemia, plasmocytoma, lid tumour, prostate cancer (= prostate tumours) etc., or infectious diseases such as, for example, influenza, malaria, SARS, yellow fever, AIDS, Lyme borreliosis, leishmaniasis, anthrax, meningitis, can be treated with the pharmaceutical composition described.

[0109] The antibody-coding (modified) RNA according to the invention or a pharmaceutical composition according to the invention can likewise be used for treatment of, for example, viral infectious diseases chosen from, without being limited thereto, AIDS, condyloma acuminata, molluscum contagiosum, dengue fever, three-day fever, Ebola virus, colds, early summer meningoencephalitis (ESME), influenza, shingles, hepatitis, herpes simplex type I, herpes simplex type II, herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, measles, foot and mouth disease, mononucleosis, mumps, Norwalk virus infection, Pfeiffer's glandular fever, smallpox, polio (poliomyelitis), pseudocroup, infectious erythema, rabies, warts, West Nile fever, chicken-pox, cytomegalovirus (CMV), caused by viruses chosen from, without being limited thereto, e.g. HIV, orthopox variola virus, orthopox alastrim virus, parapox ovis virus, molluscum contagiosum virus, herpes simplex virus 1, herpes simplex virus 2, herpes B virus, varicella zoster virus, pseudorabies virus, human cytomegaly virus, human herpes virus 6, human herpes virus 7, Epstein-Barr virus, human herpes virus 8, hepatitis B virus, chikungunya virus, O'nyong'nyong virus, rubivirus, hepatitis C virus, GB virus C, West Nile virus, dengue virus, yellow fever virus, louping ill virus, St. Louis encephalitis virus, Japan B encephalitis virus, Powassan virus, FSME virus, SARS-associated corona virus, human corona virus 229E, human corona virus Oc43, Torovirus, human T cell lymphotropic virus type I, human T cell lymphotropic virus type II, human immunodeficiency virus type 1, human immunodeficiency virus type 2, Lassa virus, lymphocytic choriomeningitis virus, Tacaribe virus, Junin virus, Machupo virus, Borna disease virus, Bunyamwera virus, California encephalitis virus, Rift Valley fever virus, sand fly fever virus, Toscana virus, Crimean-Congo haemorrhagic fever virus, Hazara virus, Khasan virus, Hantaan virus, Seoul virus, Prospect Hill virus, Puumala virus, Dobrava Belgrade virus, Tula virus, sin nombre virus, Lake Victoria Marburg virus, Zaire Ebola virus, Sudan Ebola virus, Ivory Coast Ebola virus, influenza virus A, influenza virus B, influenza viruses C, parainfluenza virus, measles virus, mumps virus, respiratory syncytial virus, human metapneumovirus, vesicular stomatitis Indiana virus, rabies virus, Mokola virus, Duvenhage virus, European bat lyssavirus 1 + 2, Australian bat lyssavirus, adenoviruses A-F, human papilloma viruses, condyloma virus 6, condyloma virus 11, polyoma viruses, adeno-associated virus 2, rotaviruses, or orbiviruses etc.,

or bacterial infectious diseases, such as abortion (infectious, septic), prostatitis (prostate inflammation), anthrax, appen-

dicitis (inflammation of the caecum), borreliosis, botulism, Campylobacter, Chlamydia trachomatis (inflammation of the urethra, conjunctiva), cholera, diphtheria, donavonosis, epiglottitis, louse-borne typhus, typhoid fever, gas gangrene, gonorrhoea, hare plague, Helicobacter pylori, whooping-cough, climatic bubo, osteomyelitis, legionnaires' disease, leprosy, listeriosis, pneumonia, meningitis, bacterial meningitis, anthrax, inflammation of the middle ear, Mycoplasma hominis, neonatal sepsis (chorioamnionitis), noma, paratyphoid fever, plague, Reiter's syndrome, Rocky Mountain spotted fever, Salmonella paratyphoid fever, Salmonella typhoid fever, scarlet fever, syphilis, tetanus, gonorrhoea, tsutsugamushi fever, tuberculosis, typhus, vaginitis (colpitis), soft chancre and infectious diseases caused by parasites, protozoa or fungi, such as amoebic dysentery, bilharziosis, Chagas' disease, Echinococcus, fish tapeworm, ichthyotoxism (ciguatera), fox tapeworm, mycosis pedis, dog tapeworm, candiosis, ptyriasis, the itch (scabies), cutaneous leishmaniasis, lamblian dysentery (giadiasis), lice, malaria, onchocercosis (river blindness), fungal diseases, beef tapeworm, schistosomiasis, sleeping sickness, pork tapeworm, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral leishmaniasis, nappy dermatitis or dwarf tapeworm.

[0110] The antibody-coding (modified) RNA according to the invention or a pharmaceutical composition according to the invention can also be used for treatment of cardiovascular diseases chosen from, without being limited thereto, coronary heart disease, arteriosclerosis, apoplexy and hypertension, and neuronal diseases chosen from Alzheimer's disease, amyotrophic lateral sclerosis, dystonia, epilepsy, multiple sclerosis and Parkinson's disease etc.

[0111] The antibody-coding (modified) RNA according to the invention or a pharmaceutical composition according to the invention can furthermore be used for treatment of autoimmune diseases chosen from, without being limited thereto, autoimmune type I diseases or autoimmune type II diseases or autoimmune type III diseases or autoimmune type IV diseases, such as, for example, multiple sclerosis (MS), rheumatoid arthritis, diabetes, diabetes type I (diabetes mellitus), systemic lupus erythematosus (SLE), chronic polyarthritis, Basedow's disease, autoimmune forms of chronic hepatitis, colitis ulcerosa, allergy type I diseases, allergy type II diseases, allergy type III diseases, allergy type IV diseases, fibromyalgia, hair loss, Bechterew's disease, Crohn's disease, myasthenia gravis, neurodermatitis, polymyalgia rheumatica, progressive systemic sclerosis (PSS), psoriasis, Reiter's syndrome, rheumatic arthritis, psoriasis, vasculitis etc., or diabetes type II.

[0112] Diseases in the context of the present invention likewise include monogenetic diseases, i.e. (hereditary) diseases which are caused by an individual gene defect and are inherited according to Mendel's rules. Monogenetic diseases in the context of the present invention are preferably chosen from the group consisting of autosomally recessive hereditary diseases, such as, for example, adenosine deaminase deficiency, familial hypercholesterolaemia, Canavan's syndrome, Gaucher's disease, Fanconi's anaemia, neuronal ceroid lipofuscinoses, mucoviscidosis (cystic fibrosis), sickle cell anaemia, phenylketonuria, alcaptonuria, albinism, hypothyroidism, galactosaemia, alpha-1 antitrypsin deficiency, xeroderma pigmentosum, Ribbing's syndrome, mucopolysaccharidoses, cleft lip, jaw, palate, Laurence-Moon-Biedl-Bardet syndrome, short rib polydactyly syndrome, cretinism, Joubert's syndrome, progeria type II, brachydactyly, adrenogenital syndrome, and X chromosomal hereditary diseases, such as, for example, colour blindness, e.g. red-green blindness, fragile X syndrome, muscular dystrophy (Duchenne and Becker-Kiener type), haemophilia A and B, G6PD deficiency, Fabry's disease, mucopolysaccharidosis, Norrie's syndrome, retinitis pigmentosa, septic granulomatosis, X-SCID, ornithine transcarbamylase deficiency, Lesch-Nyhan syndrome, or from autosomally dominant hereditary diseases, such as, for example, hereditary angioedema, Marfan's syndrome, neurofibromatosis, progeria type I, osteogenesis imperfecta, Klippel-Trenaunay syndrome, Sturge-Weber syndrome, Hippel-Lindau syndrome and tuberous sclerosis. RNA according to the invention which encodes an antibody as described here can be used on monogenetic diseases in the context of the present invention, the coded antibody being able to intervene in a regulatory manner, and also as a therapy, for example by regulation of undesirable metabolism products, trapping of specific gene products, interference with undesired certain interactions of proteins, e.g. inhibiting certain undesired ligand/receptor interactions etc.

[0113] A (modified) RNA according to the invention which encodes an antibody can be employed in various ways for treatment of the abovementioned indications. Thus, cancer diseases, for example, can be treated by immunotherapy in addition or as an alternative to known therapies. For this, for example, an RNA according to the invention which codes for a bispecific antibody can be employed, the antibody recognizing on the one hand a surface antigen, such as e.g. CD3, on T cells and on the other hand a tumour antigen, such as e.g. Her2/neu, C20, EGFR or CA-125. As a result, T cells which are positive in respect of certain surface antigens and tumour cells which express the tumour antigen are brought spatially close, which improves the recognition of the tumour cells by the immune system and therefore increases the destruction of the tumour cells.

[0114] Furthermore, e.g. in cardiac infarction cases, for example, it is possible to employ an RNA according to the invention which codes for a bispecific antibody which recognizes on the one hand a stem cell antigen, such as e.g. CD45, and on the other hand an antigen of the target tissue, such as e.g. myosin light chain, in order to increase the concentration of stem cells in the heart muscle (see also Reusch et al. Anti-CD3 x anti-epidermal growth factor receptor (EGFR) bispecific antibody redirects T-cell cytolytic activity to EGFR-positive cancers in vitro and in an animal model. Clin Cancer Res. 2006).

[0115] Furthermore, by using RNA according to the invention which codes bispecific antibodies, e.g. two different cell

types can be brought into contact or spatially close by the coded antibodies. This is advantageous, for example, in order to concentrate a cell in a tissue or to bring two proteins, e.g. antigens, into contact with or spatially close to one another, e.g. ligand and receptor or proteins which must dimerize/oligomerize in order to become activated.

[0116] RNAs according to the invention as described here which code for intrabodies can also be employed for use on the abovementioned diseases, in particular infectious diseases, autoimmune diseases and neuronal diseases and also on monogenetic diseases. Thus, intrabodies can be used in order to inhibit, as e.g. bispecific intracellularly expressed antibodies, cytoplasmic proteins (be it proteins originating from the pathogenic organism or be it proteins from the host organism), as described above. For example, RNAs according to the invention which code for intrabodies can be employed in order to inhibit IL-2R (receptor of IL-2) or ErbB2 (Her2/neu) by the coded antibodies. RNAs according to the invention which code for intrabodies are also suitable for use on virus diseases, such as e.g. HIV-1. It has furthermore been possible to demonstrate e.g. that infection of mice with scrapie, a prion disease, can be prevented by expression of an scFv fragment against the prion protein (Vertrugno et al., KDEL-tagged anti-prion intrabodies impair PrP lysosomal degradation and inhibit scrapie infectivity., Biochem Biophys Res Commun. 2005; Marasco WA, Intrabodies: turning the humoral immune system outside in for intracellular immunization, Gene Therapy (1997) 4: 11-15). RNAs according to the invention which code for intrabodies can furthermore be employed in order to bind and preferably to neutralize, by the coded antibodies, intracellularly expressed factors as described here, such as e.g. antigens, nucleic acids etc. (see above).

[0117] In this connection, the invention therefore also provides the use of an antibody-coding (modified) RNA according to the invention or of a pharmaceutical composition according to the invention, e.g. a passive vaccine according to the invention, for treatment of indications and diseases described here. This also includes, in particular, the use of the antibody-coding (modified) RNA according to the invention for passive immunization and, respectively, the use of the pharmaceutical composition described according to the invention as a passive vaccine. The use of the antibody-coding (modified) RNA according to the invention for the preparation of a pharmaceutical composition or a passive vaccine, as described here, for treatment of the indications described here is likewise included. The use of the antibody-coding (modified) RNA according to the invention for the preparation of a passive vaccine, as described here, for passive immunization against the abovementioned indications is also included.

[0118] In this connection, the invention therefore likewise provides the use of an antibody-coding (modified) RNA according to the invention, of the antibody thereby coded, of the pharmaceutical composition described here or of the passive vaccine according to the invention for therapeutic use or for inhibition/neutralization of a protein function in one of the indications described here. In this context, a protein function is preferably suppressed (neutralizing antibodies). In principle, any of the antibodies coded by the RNA according to the invention and described here simultaneously also has a neutralizing action by binding of its specific substrate. Examples include e.g. anti-CD4 antibodies for prevention of rejection of transplants, Avastin (see above), Herceptin (see above) etc.

[0119] In this connection, the invention therefore also furthermore provides the use of an antibody-coding (modified) RNA according to the invention, of the antibody thereby coded or of the pharmaceutical composition described here for therapeutic use for passive immunization by triggering an immunological effector function in the sense of a monoclonal antibody. In this context, e.g. therapy of tumour cells or pathogens, such as viruses or bacteria, in the indications as described here is rendered possible by expression and secretion of the antibody or antibody fragment. Hereby, the immune defense of the host is supported by the inventive RNA by triggering the innate, cellular or humoral immune system. Antibodies may be directed against immune suppressing factors or they may simulate the function of certain immunologically active cytokines by e.g. activating cytokine receptors.

[0120] Furthermore, in this connection an antibody-coding (modified) RNA according to the invention or the pharmaceutical composition according to the invention described here or the passive vaccine according to the invention can also be used as an immunosuppressant in the indications described above. For example, it has been possible to demonstrate that it was possible for antibodies against the CD40 ligand (CD154) or against CD3 to prevent or reduce the rejection of transplants. Such (modified) RNAs according to the invention which encode an antibody, the coded antibodies of which can bind to surface antigens or generally to surface factors of cells, such as e.g. MHC class I molecules, MHC class II molecules, T cell receptors, LMP2 molecules, LMP7 molecules, CD1, CD2, CD3, CD4, CD8, CD11, CD28, CD30, CD31, CD40, CD50, CD54, CD56, CD58, CD80, CD86, CD95, CD153, CD154, CD178, CD3 = TCR (T cell receptor) etc. are therefore preferably employed for use as immunosuppressants.

[0121] In this connection, the invention also additionally provides the use of an antibody-coding (modified) RNA according to the invention or of the pharmaceutical composition described here for therapeutic use for expansion of (certain) cells *in vitro* or *in vivo.* For example, CD4- and CD25-positive cells and regulatory T cells can be stimulated to expansion by expression of the superantagonistic CD28 antibody. Regulatory T cells which can be multiplied by expression of the superantagonistic CD28 antibody play a role above all in autoimmune diseases (Beyersdorf N, Hanke T, Kerkau T, Hunig T. Superagonistic anti-CD28 antibodies: potent activators of regulatory T cells for the therapy of autoimmune diseases. Ann Rheum Dis. 2005 Nov;64).

[0122] An antibody-coding (modified) RNA according to the invention or the pharmaceutical composition described

here can likewise be used on rheumatoid arthritis for prevention of inflammation reactions by antibodies against e.g. TNFα or other factors exacerbating the undesired immune response against e.g. the patients' proteins, as for the treatment of autoimmune diseases.

[0123] A (modified) RNA according to the invention which encodes anti-CD18 antibodies or the pharmaceutical composition described here or the passive vaccine according to the invention can furthermore also be used for reduction of inflammations by inhibition of leukocytes, e.g. in the abovementioned indications.

[0124] The present invention furthermore also provides a method for treatment and/or prevention of the above-mentioned diseases and, respectively, for (preventive) passive immunization against the abovementioned diseases, which comprises administration of the pharmaceutical composition according to the invention described, the passive vaccine according to the invention or, respectively, the RNA according to the invention to a patient, in particular a human. Such methods also relate to treatment of indications which are connected with the intra- and extracellular processes described above, with neutralization functions of antibodies, the abovementioned inhibition of certain (cell) functions by antibodies etc.

[0125] The present invention also provides an in *vitro* transcription method for the preparation of an antibody-coding (modified) RNA, comprising the following steps:

a) provision of a nucleic acid, in particular a cDNA, which codes for an antibody as described above;
b) addition of the nucleic acid, in particular a cDNA, which codes for an antibody to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, a nucleic acid mix comprising one or more optionally modified nucleotides as described above in exchange for one or more of the naturally occurring nucleotides A, G, C or U, and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged, and optionally an RNase inhibitor;
c) incubation of the nucleic acid, in particular a cDNA, which codes for an antibody in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give an antibody-coding optionally modified RNA according to the invention;
d) optionally purification of the antibody-coding (modified) RNA according to the invention and removal of the non-incorporated nucleotides from the *in vitro* transcription medium.

[0126] A nucleic acid as described in step a) of the *in vitro* transcription method according to the invention can be any nucleic acid as described here (for example single- or double-stranded DNA, cDNA etc.) which encodes an antibody as described here. DNA sequences, e.g. genomic DNA or fragments thereof, or plasmids which encode an antibody as described here, preferably in linearized form, are typically employed for this. The *in vitro* transcription can conventionally be carried out using a vector which has an RNA polymerase binding site. Any (expression) vectors known in the prior art, e.g. commercially obtainable (expression) vectors, can be used for this. Preferred (expression) vectors are, for example, those which have an SP6 or a T7 or T3 binding site upstream and/or downstream of the cloning site. The nucleic acid sequences used can thus be transcribed later as desired, depending on the RNA polymerase chosen. A nucleic acid sequence which is used for the *in vitro* transcription and codes for an antibody as described here is typically cloned into the (expression) vector, e.g. via a multiple cloning site of the vector used. Before the transcription, the (expression) vector is typically cleaved with restriction enzymes at the site at which the future 3' end of the RNA is to be found, using a suitable restriction enzyme, and the fragment is purified. This excludes the transcribed RNA from containing vector sequences, and an RNA transcript of defined length is obtained. In this context, preferably no restriction enzymes which generate overhanging ends (such as e.g. Aat II, Apa I, Ban II, Bgl I, Bsp 1286, BstX I, Cfo I, Hae II, HgiA I, Hha I, Kpn I, Pst I, Pvu I, Sac I, Sac II, Sfi I, Sph I etc.) are used. Should such restriction enzymes nevertheless be used, the overhanging 3' end is preferably filled up, e.g. with Klenow or T4 DNA polymerase.

[0127] As an alternative for step a) the nucleic acid can also be prepared as a transcription template by a polymerase chain reaction (PCR). For this, one of the primers used typically contains the sequence of an RNA polymerase binding site. Furthermore, the 5' end of the primer used preferably contains an extension of about 10 - 50 further nucleotides, more preferably of from 15 to 30 further nucleotides and most preferably of about 20 nucleotides.

[0128] Before the *in vitro* transcription, the nucleic acid, e.g. the DNA or cDNA, template is typically purified and freed from RNase in order to ensure a high yield. Purification can be carried out with the aid of any method known in the prior art, for example using a caesium chloride gradient, ion exchange methods or by purification via agarose gel electrophoresis.

[0129] According to method step b), the nucleic acid (used as the transcription template) is added to an *in vitro* transcription medium. A suitable *in vitro* transcription medium initially comprises a nucleic acid as provided under step a), for example about 0.1 - 10 μg, preferably about 1 - 5 μg, more preferably 2,5 μg and most preferably about I μg of such a nucleic acid. A suitable *in vitro* transcription medium furthermore optionally comprises a reducing agent, e.g. DTT, more preferably about 1-20 μl 50 mM DTT, even more preferably about 5 μl 50 mM DTT. The *in vitro* transcription medium furthermore comprises nucleotides, e.g. a nucleotide mix, in the case of the present invention comprising a

mixture of (modified) nucleotides as defined here (typically about 0.1-10 mM per nucleotide, preferably 0.1 to 1 mM per nucleotide (preferably about 4 mM in total)) and optionally non-modified nucleotides. If modified nucleotides as defined here (about 1 mM per nucleotide, preferably about 4 mM in total), e.g. pseudouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate etc., are employed, they are typically added in an amount such that the modified or base-modified nucleotides is completely replaced by the natural nucleotide. However, it is also possible to employ mixtures of one or more modified or base-modified nucleotides and one or more naturally occurring nucleotides instead of a particular nucleotide, i.e. it is thus possible to employ one or more modified nucleotides as described above in exchange for one or more of the naturally occurring nucleotides A, G, C or U, and optionally additionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged. Conversely, it is also possible to use only natural nucleotides. By selective addition of the desired nucleotide to the *in vitro* transcription medium the content, i.e. the occurrence and the amount, of the desired modification of nucleotides in the transcribed antibody-coding (modified) RNA sequence can therefore be controlled. A suitable *in vitro* transcription medium likewise comprises an RNA polymerase, e.g. T7 RNA polymerase (for example T7-Opti mRNA Kit, CureVac, Tübingen, Germany), T3 RNA polymerase or SP6, typically about 10 to 500 U, preferably about 25 to 250 U, more preferably about 50 to 150 U, and most preferably about 100 U of RNA polymerase. The *in vitro* transcription medium is furthermore preferably kept free from RNase in order to avoid degradation of the transcribed RNA. A suitable *in vitro* transcription medium therefore optionally additionally comprises an RNase inhibitor.

[0130] The nucleic acid is incubated in the *in vitro* transcription medium in a step c) and is transcribed to an antibody-coding (modified) RNA. The incubation times are typically about 30 to 240 minutes, preferably about 40 to 120 minutes and most preferably about 90 minutes. The incubation temperatures are typically about 30-45 °C, preferably 37-42 °C. The incubation temperature depends on the RNA polymerase used, e.g. for T7 RNA polymerase it is about 37 °C. The RNA obtained by the transcription is preferably an mRNA. The yields obtained in the *in vitro* transcription are, for the stated starting amounts employed in step b), typically in the region of about 30 μg of RNA per μg of template DNA used. In the context of the present invention, the yields obtained in the *in vitro* transcription can be increased by linear up scaling. For this, the stated starting amounts employed in step b) are preferably increased according to the yields required, e.g. by a multiplication factor of 5, 10, 50, 100, 500, 1,000, 5,000, 10,000, 50,000, 100,000 etc.

[0131] After the incubation, a purification of the transcribed antibody-coding (modified) RNA can optionally take place in step d) of the *in vitro* transcription method according to the invention. Any suitable method known in the prior art, e.g. chromatographic purification methods, e.g. affinity chromatography, gel filtration etc., can be used for this. By the purification, non-incorporated, i.e. excess nucleotides and template DNA can be removed from the *in vitro* transcription medium and a clean (modified) RNA can be obtained. For example, after the transcription the reaction mixture containing the transcribed RNA can typically be digested with DNase in order to remove the DNA template still contained in the reaction mixture. The transcribed RNA can be subsequently or alternatively precipitated with LiCl. Purification of the transcribed RNA can then take place via IP RP-HPLC. This renders it possible in particular to separate longer and shorter fragments from one another effectively.

[0132] Preferably, in this context the purification takes place via a method for purification of RNA on a preparative scale, which is distinguished in that the RNA is purified by means of HPLC using a porous reverse phase as the stationary phase (PURE Messenger). For example, for the purification in step d) of the *in vitro* method according to the invention, a reverse phase can be employed as the stationary phase for the HPLC purification. For the chromatography with reverse phases, a non-polar compound typically serves as stationary phase, and a polar solvent, such as mixtures of water, which is usually employed in the form of buffers, with acetonitrile and/or methanol, serves as the mobile phase for the elution. Preferably, the porous reverse phase has a particle size of 8.0 ± 2 μm, preferably ±1 μm, more preferably +/- 0.5 μm. The reverse phase material can be in the form of beads. The purification can be carried out in a particularly favourable manner with a porous reverse phase having this particle size, optionally in the form of beads, particularly good separation results being obtained. The reverse phase employed is preferably porous since with stationary reverse phases which are not porous, such as are described e.g. by Azarani A. and Hecker K.H., pressures which are too high are built up, so that preparative purification of the RNA is possible, if at all, only with great difficulty. The reverse phase preferably has a pore size of from 200 Å to 5,000 Å, in particular a pore size of from 300 Å to 4,000 Å. Particularly preferred pore sizes for the reverse phases are 200 - 400 Å, 800 - 1,200 Å and 3,500 - 4,500 Å. With a reverse phase having these pore sizes, particularly good results are achieved in respect of the purification of the RNA in process step d). The material for the reverse phase is preferably a polystyrene-divinylbenzene, and non-alkylated polystyrene-divinylbenzenes can be employed in particular. Stationary phases with polystyrene-divinylbenzene are known per se. For the purification in method step d), the polystyrene-divinylbenzenes which are known per se and already employed for HPLC methods and are commercially obtainable can be used. A non-alkylated porous polystyrene-divinylbenzene which in particular has a particle size of 8.0 ± 0.5 μm and a pore size of 250 - 300 Å, 900 - 1,100 Å or 3,500 - 4,500 Å is very particularly preferably used for the purification in method step d). The advantages described above can be achieved in a particularly favourable manner with this material for the reverse phases. The HPLC purification can be carried out by the ion pair method, an ion having a positive charge being added to the mobile phase as a counter-ion to the negatively

charged RNA. An ion pair having a lipophilic character, which is slowed down by the non-polar stationary phase of the reverse phase system, is formed in this manner. In practices, the precise conditions for the ion pair method must be worked out empirically for each concrete separation problem. The size of the counter-ion, its concentration and the pH of the solution contribute greatly towards the result of the separation. In a favourable manner, alkylammonium salts, such as triethylammonium acetate and/or tetraalkylammonium compounds, such as tetrabutylammonium, are added to the mobile phase. Preferably, 0.1 M triethylammonium acetate is added and the pH is adjusted to about 7. The choice of mobile phase depends on the nature of the desired separation. This means that the mobile phase found for a specific separation, such as can be known, for example, from the prior art, cannot be transferred readily to another separation problem with adequate prospect of success. The ideal elution conditions, in particular the mobile phase used, must be determined for each separation problem by empirical experiments. A mixture of an aqueous solvent and an organic solvent can be employed as the mobile phase for elution of the RNA by the HPLC method. In this context, it is favourable if a buffer which has, in particular, a pH of about 7, for example 6.5 - 7.5, e.g. 7.0, is used as the aqueous solvent; preferably, the buffer triethylammonium acetate is used, particularly preferably a 0.1 M triethylammonium acetate buffer which, as described above, also acts as a counter-ion to the RNA in the ion pair method. The organic solvent employed in the mobile phase can be acetonitrile, methanol or a mixture of these two, very particularly preferably acetonitrile. The purification of the RNA in method step d) using an HPLC method as described is carried out in a particularly favourable manner with these organic solvents. The mobile phase is particularly preferably a mixture of 0.1 M triethylammonium acetate, pH 7, and acetonitrile. It has emerged to be likewise particularly favourable if the mobile phase contains 5.0 vol.% to 20.0 vol.% of organic solvent, based on the mobile phase, and the remainder to make up 100 vol.% is the aqueous solvent. It is very particularly favourable for the method according to the invention if the mobile phase contains 9.5 vol.% to 14.5 vol.% of organic solvent, based on the mobile phase, and the remainder to make up 100 vol.% is the aqueous solvent. Elution of the RNA can subsequently be carried out isocratically or by means of a gradient separation. In the case of an isocratic separation, elution of the RNA is carried out with a single eluting agent or a mixture of several eluting agents which remains constant, it being possible for the solvents described above in detail to be employed as the eluting agent.

[0133] The present invention also provides an *in vitro* transcription and translation method for expression of an antibody, comprising the following steps:

a) provision of a nucleic acid, in particular a cDNA, which encodes an antibody as described above;
b) addition of the nucleic acid to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, a nucleic acid mix comprising one or more (modified) nucleotides as described above in exchange for one or more of the naturally occurring nucleotides A, G, C or U, and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged, and optionally an RNase inhibitor;
c) incubation of the nucleic acid, in particular a cDNA, in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give an antibody-coding (modified) RNA according to the invention;
d) optionally purification of the antibody-coding (modified) RNA according to the invention and removal of the non-incorporated nucleotides from the *in vitro* transcription medium;
e) addition of the (modified) RNA obtained in step c) (and optionally in step d) to an *in vitro* translation medium;
f) incubation of the (modified) RNA in the *in vitro* translation medium and *in vitro* translation of the antibody coded by the (modified) RNA;
g) optionally purification of the antibody translated in step f).

[0134] Steps a), b), c) and d) of the *in vitro* transcription and translation method according to the invention for expression of an antibody are identical to steps a), b), c) and d) of the *in vitro* transcription method according to the invention described here.

[0135] In step e) of the *in vitro* transcription and translation method according to the invention for expression of an antibody, the (modified) RNA transcribed in step c) (and optionally purified in step d) is added to a suitable *in vitro* translation medium. A suitable *in vitro* translation medium comprises, for example, reticulocyte lysate, wheat germ extract etc. Such a medium conventionally furthermore comprises an amino acid mix. The amino acid mix typically comprises (all) naturally occurring amino acids and optionally modified amino acids, e.g. ^{35}S-methionine (for example for monitoring the translation efficiency via autoradiography). A suitable *in vitro* translation medium furthermore comprises a reaction buffer. *In vitro* translation media are described, for example, in Krieg and Melton (1987) (P. A. Krieg and D. A. Melton (1987) In vitro RNA synthesis with SP6 RNA polymerase; Methods Enzymol 155:397-415), the disclosure content of which in this respect is included in its full scope in the present invention.

[0136] In a step f) of the *in vitro* transcription and translation method according to the invention for expression of an antibody, the (modified) nucleic acid is incubated in the *in vitro* translation medium and the antibody coded by the (modified) nucleic acid is translated *in vitro*. The incubation typically lasts about 30 to 240 minutes, preferably about 40 to 120 minutes and most preferably about 90 minutes. The incubation temperature is typically in a range of about 20-40

°C, preferably about 25 to 35 °C and most preferably about 30 °C.

[0137] Steps b) to f) of the in *vitro* transcription and translation method according to the invention for expression of an antibody or individual steps of steps b) to f) can be combined with one another, i.e. can be carried out together. In this context, all the necessary components are preferably added to the reaction medium together at the start or successively during the reaction in accordance with the sequence of the steps b) to f) described.

[0138] The translated antibody obtained in step f) can be purified in an optional step g). A purification can be carried out with methods which are known to a person skilled in the art from the prior art, e.g. chromatography, such as, for example, affinity chromatography (HPLC, FPLC, etc.), ion exchange chromatography, gel chromatography, size exclusion chromatography, gas chromatography, or antibody detection, or biophysical methods, such as e.g. NMR analyses, etc. (see e.g. Maniatis *et al.* (2001) *supra*). Chromatography methods, including affinity chromatography methods, can employ tags in a suitable manner for the purification, as described above, e.g. a hexahistidine tag (His tag, polyhistidine tag), a streptavidin tag (Strep tag), an SBP tag (streptavidin-binding tag), a GST (glutathione S transferase) tag etc. The purification can furthermore be carried out via an antibody epitope (antibody-binding tag), e.g. a Myc tag, an Swa11 epitope, a FLAG tag, an HA tag etc., i.e. via recognition of the epitope via a corresponding (immobilized) antibody. The purification can likewise be carried out via the immobilized substrate of the specific antibody, i.e. by binding of the antibody to an immobilized antigen which is recognized and, respectively, bound specifically by the translated antibody.

[0139] The present invention also provides an *in vitro* transcription and translation method for expression of an antibody in a host cell, comprising the following steps:

a) provision of a nucleic acid, in particular a cDNA, which encodes an antibody as described above;
b) addition of the nucleic acid to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, one or more (modified) nucleotides as described above in exchange for one or more of the naturally occurring nucleotides A, G, C or U and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged;
c) incubation of the nucleic acid, in particular a cDNA, in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give an antibody-coding (modified) RNA according to the invention;
d) optionally purification of the antibody-coding (modified) RNA according to the invention and removal of the non-incorporated nucleotides from the *in vitro* transcription medium;
e') transfection of the (modified) RNA obtained in step c) (and optionally d)) into a host cell;
f) incubation of the (modified) nucleic acid in the host cell and translation of the antibody coded by the (modified) RNA in the host cell;
g') optionally isolation and/or purification of the antibody translated in step f').

[0140] Steps a), b), c) and d) of the *in vitro* transcription and translation method according to the invention for expression of an antibody in a host cell are identical to steps a), b), c) and d) of the *in vitro* transcription method according to the invention described here and of the *in vitro* transcription and translation method according to the invention described here for expression of an antibody.

[0141] According to step e') of the *in vitro* transcription and translation method according to the invention, transfection of the (modified) RNA obtained in step c) (and optionally in step d)) into a host cell is carried out. The transfection is in general carried out via transfection methods known in the prior art (see, for example, Maniatis et al. (2001) Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Suitable transfection methods in the context of the present invention include, without being limited thereto, e.g. electroporation methods, including modified electroporation methods (e.g. nucleofection), calcium phosphate methods, e.g. the calcium coprecipitation method, the DEAE-dextran method, the lipofection method, e.g. the transferrin-mediated lipofection method, polyprene transfection, particle bombardment, nanoplexes, e.g. PLGA, polyplexes, e.g. PEI, protoplast fusion and the microinjection method, the lipofection method having emerged in particular as a suitable method. In this context, the (modified) RNA according to the invention can be in the naked or complexed form, as described above for the (modified) RNA according to the invention.

[0142] In connection with the present invention and with step e') of the *in vitro* transcription and translation method according to the invention for expression of an antibody in a host cell, a (suitable) host cell includes any cell which allows expression of the antibody coded by the (modified) RNA according to the invention, preferably any cultured eukaryotic cell (e.g. yeast cells, plant cells, animal cells and human cells) or prokaryotic cell (e.g. bacteria cells etc.). Cells of multicellular organisms are preferably chosen for expression of the antibody coded by the (modified) RNA according to the invention if posttranslational modifications, e.g. glycosylation of the coded protein, are necessary (N- and/or O-coupled). In contrast to prokaryotic cells, such (higher) eukaryotic cells render possible posttranslational modification of the protein synthesized. The person skilled in the art knows a large number of such higher eukaryotic cells or cell lines, e.g. 293T (embryonal kidney cell line), HeLa (human cervix carcinoma cells), CHO (cells from the ovaries of the Chinese hamster) and further cell lines, including such cells and cell lines developed for laboratory purposes, such as, for example,

hTERT-MSC, HEK293, Sf9 or COS cells. Suitable eukaryotic cells furthermore include cells or cell lines which are impaired by diseases or infections, e.g. cancer cells, in particular cancer cells of any of the types of cancer mentioned here in the description, cells impaired by HIV, and/or cells of the immune system or of the central nervous system (CNS). Human cells or animal cells, e.g. of animals as mentioned here, are particularly preferred as eukaryotic cells. Suitable host cells can likewise be derived from eukaryotic microorganisms, such as yeast, e.g. Saccharomyces cerevisiae (Stinchcomb et al., Nature, 282:39, (1997)), Schizosaccharomyces pombe, Candida, Pichia, and filamentous fungi of the genera Aspergillus, Penicillium, etc. Suitable host cells likewise include prokaryotic cells, such as e.g. bacteria cells, e.g. from Escherichia coli or from bacteria of the genera Bacillus, Lactococcus, Lactobacillus, Pseudomonas, Streptomyces, Streptococcus, Staphylococcus, preferably E. coli, etc.

[0143] In step f) of the *in vitro* transcription and translation method according to the invention for expression of an antibody in a host cell, incubation of the (modified) RNA in the host cell and translation of the antibody coded by the (modified) RNA in the host cell are carried out. Expression mechanisms intrinsic to the host cell are preferably used for this, e.g. by translation of the RNA in the host cell via ribosomes and tRNAs. The incubation temperatures used in this context depend on the particular host cell systems used.

[0144] In an optional step g'), the translated antibody obtained in step f) can be isolated and/or purified. In this context, an isolation of the translated (expressed) antibody typically comprises separating off the antibody from reaction constituents, and can be carried out by methods which are known to a person skilled in the art, for example by cell lysis, breakdown by ultrasound, or similar methods, including the abovementioned methods. A purification can therefore also be carried out by methods as described for step e) of the *in vitro* transcription and translation method according to the invention for expression of an antibody.

[0145] For purification of (recombinant) antibodies from a host cell in step g') of the method described above, a different choice of the host cells described above is necessary, depending on the use. Thus, the production of recombinant antibodies in E. coli typically is possible to only a limited extent, since the antibodies coded by a (modified) RNA according to the invention are very complex, require complicated folding mechanisms and are conventionally modified posttranslationally for use in multicellular organisms or beings. These mechanisms conventionally cannot be implemented in the cytoplasm of E. coli. Periplasmic production in E. coli, in which correct folding and modification of the antibody fragments is possible, can therefore be used. In this context, the purification usually requires an involved breakdown of the bacteria and a difficult separating off of all the bacterial constituents which can act as endotoxins during a therapeutic use. To bypass these purification problems, expression systems for yeasts, insect cells, mammalian cells and plants can be employed according to the invention in such cases, the production preferably being carried out in suitable mammalian cells, such as e.g. hamster cells (CHO), as described here.

[0146] Regardless of steps (a) to (d), the antibody coded by the (modified) RNA according to the invention can also be expressed by an *in vitro* translation method of steps (e') to (g'), which is also subject matter of the present invention as such.

[0147] The present invention also provides an *in vitro* transcription and *in vivo* translation method for expression of an antibody in an organism, comprising the following steps:

a) provision of a nucleic acid, in particular a cDNA, which encodes an antibody as described above;
b) addition of the nucleic acid to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, a nucleic acid mix comprising one or more (modified) nucleotides as described above in exchange for one or more of the naturally occurring nucleotides A, G, C or U, and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged, and optionally an RNase inhibitor;
c) incubation of the nucleic acid, in particular a cDNA, in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give a (modified) RNA according to the invention as described here;
d) optionally purification and removal of the non-incorporated nucleotides from the *in vitro* transcription medium;
e") transfection of the (modified) RNA obtained in step c) (and optionally in step d)) into a host cell and transplanting of the transfected host cell into an organism;
f") translation of the antibody coded by the (modified) RNA in the organism.

[0148] Steps a), b), c) and d) of the *in vitro* transcription and *in vivo* translation method according to the invention for expression of an antibody in an organism are identical to steps a), b), c) and d) of the *in vitro* transcription method according to the invention described here, of the *in vitro* transcription and translation method according to the invention described here for expression of an antibody and of the *in vitro* transcription and translation method according to the invention described here for expression of an antibody in a host cell.

[0149] Host cells in the context of the present invention, and in particular in step e"), can also include autologous cells, i.e. cells which are taken from a patient and returned again (endogenous cells). Such autologous cells reduce the risk of rejection by the immune system in the case of *in vivo* uses. In the case of autologous cells, (healthy or diseased) cells from the affected body regions/organs of the patient are preferably employed. Transfection methods are preferably those

as described above for step e). In step e"), transplanting of the host cell into an organism is carried out, additionally to step e). An organism or a being in connection with the present invention typically means mammals, i.e. animals, including cattle, pig, dog, cat, donkey, monkey, including rodents, e.g. mouse, hamster, rabbit etc., and humans. As an alternative to step e") and f"), the isolation and/or purification can be carried out according to steps f)/f') and/or g)/g') and the translated (therapeutically active) protein can be administered subsequently to the being. The administration can be carried out as described for pharmaceutical compositions.

[0150] In step f"), translation of the antibody coded by the (modified) RNA is carried out in the organism. In this context, the translation is preferably carried out by means of systems specific to the host cell, depending on the host cell used.

[0151] Regardless of steps (a) to (d), the transcribed (modified) RNA according to the invention can also be expressed by an *in vitro* translation method of steps (e") to (g"), which is also subject matter of the present invention as such.

[0152] As an alternative to the methods described above, according to a particularly preferred embodiment in a further step e'") a (modified) RNA according to the invention transcribed according to steps a) to d) can be administered directly into the organism, e.g. human, e.g. by administration of the naked or complexed RNA according to the invention, for example using the transfection methods described above, optionally using certain stabilizing factors described here. In this context, after uptake the RNA is preferably transported into the cells, e.g. with localization or signal sequences as described here, and preferably translated into the coded antibody in the cells.

Advantages of the invention

[0153] The present invention describes in particular an antibody-coding RNA according to the invention. This can be modified or non-modified, where the definition of "modification" is to be understood in the broadest sense. A native RNA covalently bonded to another group, for example a lipid or a sugar residue, is modified in the context of this invention. An RNA which contains non-natively occurring constituents, for example non-native nucleotides, or an RNA which is modified with respect to its precursor by exchange of nucleotides, regardless of whether these are native or non-native, is also modified in the context of the invention. The great advantage of such RNAs is that these do not have the negative actions of DNA transfections (with stable incorporation into the genome). In the case of modified antibody-coding RNAs, the limited stability of the RNA coding for the antibodies or antibody fragments is moreover improved. According to the invention, after administration to patients, in particular mammals, above all humans, the antibodies are therefore thus expressed *in vivo* for only an estimatable time beyond the treatment and therefore do not lead to harmful effects. In contrast, the conventional intrabody DNAs can be integrated into the genome in a stable manner or at least expressed persistently, which can lead to uncontrollable events. The great advantage compared with administration of monoclonal antibodies *in vivo* is furthermore that with the use of an antibody-coding (modified) RNA as described here, no antibodies have to be prepared and purified in an involved manner and they are therefore considerably less expensive to prepare. The most essential advantage of the present invention is, however, that intracellularly expressed proteins can also be achieved with the antibodies coded by (modified) RNAs according to the invention, which is not possible with monoclonal antibodies known hitherto from the prior art.

[0154] The following figures and examples are intended to explain in more detail and illustrate the above description, without being limited thereto.

Figures:

[0155]

Figure 1 illustrates the structure of an IgG antibody. IgG antibodies are built up from in each case two identical light and two heavy protein chains which are bonded to one another via disulfide bridges. The light chain comprises the N-terminal variable domain V_L and the C-terminal constant domain C_L. The heavy chain of an IgG antibody can be divided into an N-terminal variable domain V_H and three constant domains C_H1, C_H2 and C_H3.

Figure 2 shows the gene cluster for the light and the heavy chains of an antibody: (A): Gene cluster for the light chain κ. (B): Gene cluster for the light chain λ. (C): and (D): Gene cluster for the heavy chain. In this context, the variable region of a heavy chain is composed of three different gene segments. In addition to the V and J segments, additional D segments are also found here. The V_H, D_H and J_H segments can likewise be combined with one another virtually as desired to form the variable region of the heavy chain.

Figure 3 illustrates in the form of a diagram the differences in the light and heavy chains of murine (i.e. obtained in the mouse host organism), chimeric, humanized and human antibodies.

Figure 4 shows an overview of the structure of various antibody fragments. The constituents of the antibody fragments are shown on a dark grey background.

Figure 5 shows various variants of antibodies and antibody fragments in Figure 5A, 5B and 5C:

(A) shows a diagram of an IgG antibody of two light and two heavy chains.
(B) shows an Fab fragments from the variable and a constant domain in each case of a light and a heavy chain. The two chains are bonded to one another via a disulfide bridge.
(C) shows an scFv fragment from the variable domain of the light and the heavy chain, which are bonded to one another via an artificial polypeptide linker.

Figure 6 shows a presentation of an antibody-coding (modified) RNA according to the invention as an expression construct. In this:

V_H = variable domain of the heavy chain;
C_H = constant domain of the heavy chain;
V_L = variable domain of the light chain;
C_L = constant domain of the light chain;
SIRES = internal ribosomal entry site (IRES, superIRES)
muag = mutated form of the 3' UTR of the alpha-globin gene; and
A70C30 = polyA-polyC tail.

Figure 7 shows a diagram of the detection of an antibody coded by an RNA according to the invention by means of ELISA on the example of the antigen Her2.

Figure 8 shows the wild-type DNA sequence of the heavy chain of the antibody rituximab (= Rituxan, MabThera) (wild-type: GC content: 56.5 %, length: 1,344) (SEQ ID NO: 1).

Figure 9 shows the GC-optimized DNA sequence of the heavy chain of the antibody rituximab (= Rituxan, MabThera) (GC content: 65.9 %, length: 1,344) (SEQ ID NO: 2).

Figure 10 shows the wild-type DNA sequence of the light chain of the antibody rituximab (= Rituxan, MabThera) (wild-type: GC content: 58.5 %, length: 633) (SEQ ID NO: 3).

Figure 11 shows the GC-optimized DNA sequence of the light chain of the antibody rituximab (= Rituxan, Mab-Thera) (GC content: 67.2 %, length: 633) (SEQ ID NO: 4).

Figure 12 shows the total construct of the GC-optimized DNA sequence of the antibody rituximab (= Rituxan, MabThera) with the light and heavy chains (SEQ ID NO: 5). The total construct contains the following sequences and cleavage sites (see also alternative cleavage sites of Fig. 25, SEQ ID No. 51):

ACC linker for an optimum Kozak sequence
AAGCTT HindIII
TGA stop codon
ACTAGT SpeI
AGATCT BglII
ATGCAT NsiI
CATCATCATCATCATCAT His tag
Signal peptide, HLA-A*0201: *GC-rich*

ATGGCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGACG CAGACCTGGGCCGGG.

The coding region of the heavy chain sequence starts with the signal peptide as given above (*italic*). This region is G/C enriched as well. The subsequent sequence starting with CAG represents the actual antibody coding sequence (see Fig. 9) for the heavy chain, which ends with AAG and is followed by the above described His tag sequence. Finally, the open reading frame for the heavy chain ends with the stop codon

TGA (__). The coding region for the light chain sequence starts 3' upstream with the signal peptide's ATG as given above followed by the light chain's coding region for the light chain starting with CAG running to the stop codon TGA (___) (see Figure 11). Both coding regions for the light and the heavy chain are separated by an IRES element (____). The inventive RNA coded by the construct given in Figure 12 may or may not contain a (His) tag sequence and may contain a signal peptide sequence different from the above peptide sequence or may even have no signal peptide sequence. Accordingly, the inventive RNA molecule contains preferably the coding region (with or without a signal peptide sequence at its beginning) of the heavy and/or the light chain (e.g. as shown in Figure 12), preferably in combination with at least one ribosomal entry site.

Figure 13 shows the wild-type DNA sequence of the heavy chain of the antibody cetuximab (= Erbitux) (wild-type: GC content: 56.8 %, length: 1,359) (SEQ ID NO: 6).

Figure 14 shows the GC-optimized DNA sequence of the heavy chain of the antibody cetuximab (= Erbitux) (GC content: 65.9 %, length: 1,359) (SEQ ID NO: 7).

Figure 15 shows the wild-type DNA sequence of the light chain of the antibody cetuximab (= Erbitux) (wild-type: GC content: 58.2 %, length: 642) (SEQ ID NO: 8).

Figure 16 shows the GC-optimized DNA sequence of the light chain of the antibody cetuximab (= Erbitux) (GC content: 65.7 %, length: 642) (SEQ ID NO: 9).

Figure 17 shows the total construct of the GC-optimized DNA sequence of the antibody cetuximab (= Erbitux) with the light and heavy chains (SEQ ID NO: 10). The total construct contains the following sequences and cleavage sites (see also alternative cleavage sites of Fig. 26, SEQ ID No 52):

ACC linker for an optimum Kozak sequence
AACCTT HindIII
TGA stop codon
ACTAGT SpeI
AGATCT BglII
ATGCAT NsiI
CATCATCATCATCATCAT His tag
Signal peptide, HLA-A*0201: *GC-rich*

ATGGCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGACG CAGACCTGGGCCGGG.

The coding region of the heavy chain sequence starts with the signal peptide as given above (*italic*). This region is G/C enriched as well. The subsequent sequence starting with CAG represents the actual antibody coding sequence (see Fig. 14) for the heavy chain, which ends with AAG and is followed by the above described His tag sequence. Finally, the open reading frame for the heavy chain ends with the stop codon TGA (___). The coding region for the light chain sequence starts 3' upstream with the signal peptide's ATG as given above followed by the light chain's coding region for the light chain starting with GAC running to the stop codon TGA (___) (see Figure 16). Both coding regions for the light and the heavy chain are separated by an IRES element (____). The inventive RNA coded by the construct given in Figure 17 may or may not contain a (His) tag sequence and may contain a signal peptide sequence different from the above peptide sequence or may even have no signal peptide sequence. Accordingly, the inventive RNA molecule contains preferably the coding region (with or without a signal peptide sequence at its beginning) of the heavy and/or the light chain (e.g. as shown in Figure 17), preferably in combination with at least one ribosomal entry site.

Figure 18 shows the wild-type DNA sequence of the heavy chain of the antibody trastuzumab (= Herceptin) (wild-type: GC content: 57.8 %, length: 1,356) (SEQ ID NO: 11).

Figure 19 shows the GC-optimized DNA sequence of the heavy chain of the antibody trastuzumab (= Herceptin) (GC content: 67.0 %, length: 1,356) (SEQ ID NO: 12).

Figure 20 shows the wild-type DNA sequence of the light chain of the antibody trastuzumab (= Herceptin) (wild-type: GC content: 56.9 %, length: 645) (SEQ ID NO: 13).

Figure 21 shows the GC-optimized DNA sequence of the light chain of the antibody trastuzumab (= Herceptin) (GC content: 66.4 %, length: 645) (SEQ ID NO: 14).

Figure 22 shows the total construct of the GC-optimized DNA sequence of the antibody trastuzumab (= Herceptin) with the light and heavy chains (SEQ ID NO: 15). The total construct contains the following sequences and cleavage sites (see also alternative cleavage sites of Fig. 27, SEQ ID No. 53):

ACC linker for an optimum Kozak sequence
AAGCTT HindIII
TGA stop codon
ACTAGT SpeI
AGATCT BglII
ATGCAT NsiI
CATCATCATCATCATCAT His tag
Signal peptide, HLA-A*0201: *GC-rich*

ATGGCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGACG CAGACCTGGGCCGGG.

The coding region of the heavy chain sequence starts with the signal peptide as given above (*italic*). This region is G/C enriched as well. The subsequent sequence starting with GAG represents the actual antibody coding sequence (see Fig. 19) for the heavy chain, which ends with AAG and is followed by the above described His tag sequence. Finally, the open reading frame for the heavy chain ends with the stop codon TGA (___). The coding region for the light chain sequence starts 3' upstream with the signal peptide's ATG as given above followed by the light chain's coding region for the light chain starting with GAC running to the stop codon TGA (___) (see Figure 21). Both coding regions for the light and the heavy chain are separated by an IRES element (____). The inventive RNA coded by the construct given in Figure 22 may or may not contain a (His) tag sequence and may contain a signal peptide sequence different from the above peptide sequence or may even have no signal peptide sequence. Accordingly, the inventive RNA molecule contains preferably the coding region (with or without a signal peptide sequence at its beginning) of the heavy and/or the light chain (e.g. as shown in Figure 22), preferably in combination with at least one ribosomal entry site.

Figure 23 shows RNA-mediated antibody expression in cell culture. CHO or BHK cells were transfected with 20 μg of antibody-encoding mRNA according to the invention which was produced (RNA, G/C enriched, see above) or mock-transfected. 24 hours after transfection protein synthesis was analysed by Western blotting of cell lysates. Cells harboured about 0.5 μg of protein as assessed by Western Blot analysis. Each lane represents 10% of total lysate. Humanised antibodies served as control and for a rough estimate of protein levels. The detection antibody recognises both heavy and light chains; moreover, it shows some unspecific staining with cell lysates (three distinct bands migrating much slower than those of the antibodies). A comparison with control antibodies clearly demonstrates that heavy and light chains were produced in equal amounts.

Figure 24 shows that RNA-mediated antibody expression gives rise to a functional protein (antibody). Functional antibody formation was addressed by FACS staining of antigen-expressing target cells. In order to examine the production of functional antibodies, cell culture supernatants of RNA-transfected (20 μg of Ab-RNA as defined above in Example 1) cells were collected after 48 to 96 hours. About 8% of total supernatant was used to stain target cells expressing the respective antigen. Humanised antibodies served as control and for a rough estimate of protein levels. Primary antibody used for cell staining: a) humanised antibody; b) none; c,d) supernatant from RNA-transfected cells expressing the respective antibody; e) supernatant from mock-transfected CHO cells. Calculations on the basis of the analysis shown in Fig. 24 reveal that cells secreted more than 12-15 μg of functional antibody within 48-96 hours. Accordingly, the present invention proves that RNA encoding antibodies may enter into cell, may be expressed within the cell and considerable amounts of RNA encoded antibodies are then secreted by the cell into the surrounding

medium/extracellular space. Cell transfection in vivo or in vitro by the inventive RNA may therefore be used to provide antibodies acting e.g. therapeutically in the extracellular space.

Figure 25 shows an alternative sequence of the construct of Fig. 12 (antibody rituximab), wherein the restriction sites have been modified as compared to SEQ ID No. 5 of Fig. 12 (SEQ ID No.: 51). For closer information with regard to the description of various sequence elements it is referred to Figure 12.

Figure 26 shows an alternative sequence of the construct of Fig. 17 (antibody cetuximab), wherein the restriction sites have been modified as compared to SEQ ID No. 10 of Fig. 17 (SEQ ID No.: 52). For closer information with regard to the description of various sequence elements it is referred to Figure 17.

Figure 27 shows an alternative sequence of the construct of Fig. 22 (antibody trastuzumab), wherein the restriction sites have been modified as compared to SEQ ID No. 15 of Fig. 22 (SEQ ID No.: 53). For closer information with regard to the description of various sequence elements it is referred to Figure 22.

[0156] The following examples explain the present invention in more detail, without limiting it.

Examples

1. Example

1.1 Cell lines and cell culture conditions used:

[0157] The cell lines HeLa (human cervix carcinoma cell line; Her2-positive), HEK293 (human embryonal kidney; Her2-negative) and BHK21 (Syrian hamster kidney; Her2-negative) were obtained from the DMSZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) in Braunschweig and cultured in RPMI medium enriched with 2mM L-glutamine (Bio Whittaker) and 10 μg/ml streptomycin and 10 U/ml of penicillin at 37 °C under 5 % CO_2.

1.2 Preparation of expression vectors for modified RNA sequences according to the invention:

[0158] For the production of modified RNA sequences according to the invention, the GC-enriched and translation-optimized DNA sequences which code for a heavy chain and a light chain of an antibody (e.g. cetuximab (Erbitux), trastuzumab (Herceptin) and rituximab (Rituxan), cf. SEQ ID NO: 1-15, where SEQ ID NO: 1, 3, 6, 8, 11 and 13 represent the particular coding sequences which are not GC-optimized of the heavy and the light chains of these antibodies and SEQ ID NO: 2, 4, 5, 7, 9, 10, 12, 14 and 15 represent the coding GC-enriched sequences (see above)) were cloned into the pCV19 vector (CureVac GmbH) by standard molecular biology methods. To ensure equimolar expression of the two chains, an IRES (internal ribosomal entry site) was introduced. The mutated 3' UTR (untranslated region) of the alpha-globin gene and a polyA-polyC tail at the 3' end serve for additional stabilizing of the mRNA. The signal peptide of the HLA-A*0201 gene is coded for secretion of the antibody expressed. A His tag was additionally introduced for detection of the antibody. Fig. 6 shows the expression constructs used.

1.3 Preparation of the G/C-enriched and translation-optimized antibody-coding mRNA

[0159] An *in vitro* transcription was carried out by means of T7 polymerase (T7-Opti mRNA Kit, CureVac, Tübingen, Germany), followed by purification with Pure Messenger™ (CureVac, Tübingen, Germany). For this, a DNase digestion was first carried out, followed by an LiCl precipitation and thereafter an HPLC using a porous reverse phase as the stationary phase (PURE Messenger).

1.4 Detection of RNA-antibody by means of flow cytometry:

[0160] 1 million cells were transfected with the mRNA according to one of SEQ ID NO: 5, 10 or 15 (see above), which codes for an antibody as described above, by means of electroporation and were then cultured in the medium for 16 h. The antibody expressed was detected by means of an FITC-coupled His tag antibody. Alternatively, the secreted antibody from the supernatant of transfected cells was added to non-transfected, antigen-expressing cells and, after incubation, detected by the same method.

1.5 *In vitro* detection of an antibody coded by an RNA according to the invention by means of

ELISA:

[0161] A microtitre plate was loaded with a murine antibody (1) against a first antigen (HER-2). Cell lysate of antigen-expressing cells was then added to the plate. The antigen was bound here by the murine antigen-specific antibody (1). The supernatant of cells which were transfected with a modified mRNA according to the invention which codes for an HER-2-specific antibody was then added to the microtitre plate. The HER-2-specific antibody (2) contained in the supernatant likewise binds to the antibody-bound antigen, the two antibodies recognizing different domains of the antigen. For detection of the bound antibody (2), anti-human IgG coupled to horseradish peroxidase (3-HRP) was added, the substrate TMB being converted and the result determined photometrically.

1.6 *In vivo* detection of an antibody coded by an RNA according to the invention:

[0162] An antibody-coding (m)RNA according to the invention as described above was injected intradermally or intramuscularly into BALB/c mice. 24 h thereafter, the corresponding tissues were removed and protein extracts were prepared. The expression of the antibody was detected by means of ELISA as described here.

1.7 Detection of an antibody coded by an RNA according to the invention by means of western blotting:

[0163] The expressed antibodies from the supernatant of cells which were transfected with a modified mRNA which codes for an antibody as described above were separated by means of a polyacrylamide gel electrophoresis and then transferred to a membrane. After incubation with anti-His tag antibody and a second antibody coupled to horseradish peroxidase, the antibody expressed was detected by means of chemoluminescence.

1.8 Tumour model:

[0164] SKOV-3 cells were injected subcutaneously into BALB/c mice. Within the following 28 days, eight portions of 10 μg of a modified mRNA which codes for an antibody as described above were injected into the tail vein of the mice. The tumour growth was monitored over a period of 5 weeks.

2. Example

2.1. Cell lines

[0165] RNA-based expression of humanised antibodies was done in either CHO-K1 or BHK-21 cells. The tumour cell lines BT-474, A-431 and Raji strongly expressing HER2, EGFR and CD20, respectively, were used to record antibody levels. All cell lines except CHO were maintained in RPMI supplemented with FCS and glutamine according to the supplier's information. CHO cells were grown in Ham's F12 supplemented with 10% FCS. All cell lines were obtained from the German collection of cell cultures (DSMZ).

2.2. Antibody expression

[0166] Various amounts of antibody-RNA (G/C enriched as defined by Figures 12, 17, 22, 25, 26, 27) encoding the humanised antibodies Herceptin, Erbitux, and Rituxan, respectively, (see the description given above for Example 1) were transfected into either CHO or BHK cells by electroporation. Conditions were as follows: 300 V, 450 μF for CHO and 300 V, 150 μF for BHK.. After transfection, cells were seeded onto 24-well cell culture plates at a density of 2-400.000 cells per well. For collection of secreted protein, medium was replaced by 250 μl of fresh medium after cell attachment to the plastic surface. Secreted protein was collected for 24-96 hours and stored at 4 °C. In addition, cells were harvested into 50 μl of phosphate buffered saline containing 0.5% BSA and broken up by three freeze-thaw cycles. Cell lysates were cleared by centrifugation and stored at -80 °C.

2.3. Western Blot analysis

[0167] In order to detect translation of transfected RNA, proteins from either cell culture supernatants or cell lysates were separated by a 12% SDS-PAGE and blotted onto a nitrocellulose membrane. Humanised antibodies Herceptin (Roche), Erbitux (Merck KGAA), and Mabthera = Rituxan (Roche) were used as controls. After blotting was completed, membranes were consecutively incubated with biotinylated goat anti-human IgG (Dianova), streptavidin coupled to

horseradish peroxidase (BD), and a chemiluminescent substrate (SuperSignal West Pico, Pierce). Staining was detected with a Fuji LAS-1000 chemiluminescence camera.

2.4. FACS analysis

[0168] 200.000 target cells expressing the respective antigen were incubated with either control antibodies (Herceptin, Erbitux, Mabthera) or cell culture supernatants. For detection of bound antibodies, cells were stained with biotinylated goat anti-human IgG (Dianova) and PE-labelled streptavidin (Invitrogen). Cells were analysed on a FACSCalibur (BD).

Items

[0169]

1. RNA for intracellular expression of an antibody, wherein the RNA contains at least one coding region, wherein at least one coding region codes for at least one antibody.

2. RNA according to item 1, wherein the RNA is single-stranded or double-stranded, linear or circular, in the form of rRNA, tRNA or mRNA.

3. RNA according to item 1 or 2, wherein the RNA is an mRNA.

4. RNA according to item 1 to 3, wherein the antibody coded is chosen from monoclonal and polyclonal antibodies, chimeric antibodies, human antibodies, humanized antibodies, bispecific antibodies, intrabodies and fragments of these antibodies.

5. RNA according to item 1 to 4, wherein the coded antibody fragments are chosen from Fab, Fab', $F(ab')_2$, Fc, Facb, pFc', Fd, and Fv or scFv fragments of these antibodies.

6. RNA according to item 1 to 5, wherein the coded antibodies or antibody fragments specifically recognize and bind tumour-specific surface antigens chosen from (TSSA), 5T4, $\alpha 5\beta 1$-integrin, 707-AP, AFP, ART-4, B7H4, BAGE, β-catenin/m, Bcr-abl, MN/C IX-antigen, CA125, CAMEL, CAP-1, CASP-8, β-catenin/m, CD4, CD19, CD20, CD22, CD25, CDC27/m, CD 30, CD33, CD52, CD56, CD80, CDK4/m, CEA, CT, Cyp-B, DAM, EGFR, ErbB3, ELF2M, EMMPRIN, EpCam, ETV6-AML1, G250, GAGE, GnT-V, Gp100, HAGE, HER-2/neu, HLA-A*0201-R170I, HPV-E7, HSP70-2M, HAST-2, hTERT (or hTRT), iCE, IGF-1R, IL-2R, IL-5, KIAA0205, LAGE, LDLR/FUT, MAGE, MART-1/Melan-A, MART-2/Ski, MC1R, myosin/m, MUC1, MUM-1, -2, -3, NA88-A, PAP, proteinase-3, p190 minor bcr-abl, Pml/RARα, PRAME, PSA, PSM, PSMA, RAGE, RU1 or RU2, SAGE, SART-1 or SART-3, survivin, TEL/AML1, TGFβ, TPI/m, TRP-1, TRP-2, TRP-2/INT2, VEGF and WT1, NY-Eso-1 and NY-Eso-B.

7. RNA according to one of items 1 to 6, wherein the RNA is modified.

8. RNA according to one of the preceding items, wherein the modification is chosen from modifications of the nucleotide sequence compared with a precursor RNA sequence by introduction of non-native nucleotides and/or by covalent coupling of the RNA with another group.

9. RNA according to item 8, wherein the RNA has a G/C content in the coding region of the base-modified RNA which is greater than the G/C content of the coding region of the native RNA sequence, the coded amino acid sequence being unchanged with respect to the wild-type or, respectively, the precursor RNA.

10. RNA according to item 8 or 9, wherein the coding region of the modified RNA is modified compared with the coding region of the native RNA such that at least one codon of the native RNA which codes for a tRNA which is relatively rare in the cell is exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and which carries the same amino acid as the relatively rare tRNA.

11. RNA according to one of items 8 to 10, wherein the RNA has a lipid modification.

12. RNA according to one of items 8 to 11, wherein the RNA contains on at least one nucleotide of the RNA a modification of a nucleotide, wherein the nucleotides are chosen from 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-

acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), pseudouracil, 1-methyl-pseudouracil, queosine, β-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine.

13. RNA according to one of items 8 to 12, wherein the RNA contains on at least one nucleotide of the RNA a modification of a nucleotide, wherein the nucleotides are base-modified nucleotides chosen from the group consisting of 2-amino-6-chloropurine riboside 5'-triphosphate, 2-aminoadenosine 5'-triphosphate, 2-thiocytidine 5'-triphosphate, 2-thiouridine 5'-triphosphate, 4-thiouridine 5'-triphosphate, 5-aminoallylcytidine 5'-triphosphate, 5-aminoallyluridine 5'-triphosphate, 5-bromocytidine 5'-triphosphate, 5-bromouridine 5'-triphosphate, 5-iodocytidine 5'-triphosphate, 5-iodouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate, 5-methyluridine 5'-triphosphate, 6-azacytidine 5'-triphosphate, 6-azauridine 5'-triphosphate, 6-chloropurine riboside 5'-triphosphate, 7-deazaadenosine 5'-triphosphate, 7-deazaguanosine 5'-triphosphate, 8-azaadenosine 5'-triphosphate, 8-azidoadenosine 5'-triphosphate, benzimidazole riboside 5'-triphosphate, N1-methyladenosine 5'-triphosphate, N1-methylguanosine 5'-triphosphate, N6-methyladenosine 5'-triphosphate, O6-methylguanosine 5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate.

14. RNA according to item 13, wherein the base-modified nucleotides are chosen from the group consisting of 5-methylcytidine 5'-triphosphate and pseudouridine 5'-triphosphate.

15. RNA according to one of the preceding items, wherein the RNA additionally has a 5' cap structure chosen from the group consisting of m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G.

16. RNA according to one of the preceding items, wherein the RNA additionally has a poly-A tail of from about 10 to 200 adenosine nucleotides.

17. RNA according to one of the preceding items, wherein the RNA additionally has a poly-C tail of from about 10 to 200 cytosine nucleotides.

18. RNA according to one of the preceding items, wherein the RNA additionally codes a tag for purification chosen from the group consisting of a hexahistidine tag (HIS tag, polyhistidine tag), a streptavidin tag (Strep tag), an SBP tag (streptavidin-binding tag) or a GST (glutathione S-transferase) tag, or codes for a tag for purification via an antibody epitope chosen from the group consisting of antibody-binding tags, a Myc tag, a Swa11 epitope, a FLAG tag or an HA tag.

19. RNA according to one of the preceding items, wherein the RNA additionally codes a signal peptide and/or a localization sequence, in particular a secretion sequence.

20. RNA according to item 19, wherein the localization sequence is chosen from one of the sequences according to SEQ ID NO: 18 to 50.

21. RNA according to one of the preceding items, wherein the RNA contains an antibody-coding sequence which codes for the heavy chains according to SEQ ID NO: 2 and the light chains according to SEQ ID NO: 4.

22. RNA according to one of the preceding items 1 to 20, wherein the RNA contains an antibody-coding sequence according to SEQ ID NO: 5.

23. RNA according to one of the preceding items 1 to 20, wherein the RNA contains an antibody-coding sequence which codes for the heavy chains according to SEQ ID NO: 7 and the light chains according to SEQ ID NO: 9.

24. RNA according to one of the preceding items 1 to 20, wherein the RNA contains an antibody-coding sequence according to SEQ ID NO: 10.

25. RNA according to one of the preceding items 1 to 20, wherein the modified RNA contains an antibody-coding

sequence which codes for the heavy chains according to SEQ ID NO: 12 and the light chains according to SEQ ID NO: 14.

26. RNA according to one of the preceding items 1 to 20, wherein the RNA contains an antibody-coding sequence according to SEQ ID NO: 15.

27. RNA according to one of the preceding items 1 to 20, wherein the modified RNA contains an antibody-coding sequence which has a sequence identity of at least 70 % to the sequence SEQ ID NO: 5, 10 or 15 over the total length of the nucleic acid sequence of SEQ ID NO: 5, 10 or 15.

28. Pharmaceutical composition comprising an RNA according to one of items 1 to 27.

29. Use of an RNA sequence as defined in items 1 to 27 for the preparation of a pharmaceutical composition for treatment of cancer diseases, cardiovascular diseases, infectious diseases or autoimmune diseases.

30. Use according to item 29, wherein the pharmaceutical composition is in the form of a passive vaccine for treatment of tumour or infectious diseases.

31. Use according to one of items 29 or 30, wherein the cancer diseases or tumour diseases are chosen from the group consisting of melanomas, malignant melanomas, colon carcinomas, lymphomas, sarcomas, blastomas, kidney carcinomas, gastrointestinal tumours, gliomas, prostate tumours, bladder cancer, rectal tumours, stomach cancer, oesophageal cancer, pancreatic cancer, liver cancer, mammary carcinomas (= breast cancer), uterine cancer, cervical cancer, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), hepatomas, diverse virus-induced tumours, such as e.g. papilloma virus-induced carcinomas (e.g. cervix carcinoma = cervical cancer), adenocarcinomas, herpes virus-induced tumours (e.g. Burkitt's lymphoma, EBV-induced B cell lymphoma), hepatitis B-induced tumours (hepatocell carcinomas), HTLV-1- and HTLV-2-induced lymphomas, acusticus neurinoma, lung carcinomas (= lung cancer = bronchial carcinoma), small cell lung carcinomas, throat cancer, anal carcinoma, glioblastoma, rectum carcinoma, astrocytoma, brain tumours, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, testicular cancer, thyroid carcinoma, Hodgkin's syndrome, meningeomas, Schneeberger's disease, pituitary tumour, mycosis fungoides, carcinoids, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, kidney cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumours, oligodendroglioma, vulval cancer, intestinal cancer, colon carcinoma, oesophageal carcinoma (= oesophageal cancer), wart conditions, small intestine tumours, craniopharyngeomas, ovarian carcinoma, soft tissue tumours (sarcomas), ovarian cancer (= ovarian carcinoma), pancreatic carcinoma (= pancreatic cancer), endometrium carcinoma, liver metastases, penis cancer, tongue cancer, gallbladder cancer, leukaemia, plasmocytoma, lid tumour and prostate cancer (= prostate tumours).

32. Use according to one of items 29 to 30, wherein the infectious diseases are chosen from the group consisting of influenza, malaria, SARS, yellow fever, AIDS, Lyme borreliosis, leishmaniasis, anthrax, meningitis, viral infectious diseases, such as AIDS, condyloma acuminata, molluscum contagiosum, dengue fever, three-day fever, Ebola virus, colds, early summer meningoencephalitis (ESME), influenza, shingles, hepatitis, herpes simplex type I, herpes simplex type II, herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, measles, foot and mouth disease, mononucleosis, mumps, Norwalk virus infection, Pfeiffer's glandular fever, smallpox, polio (poliomyelitis), pseuodcroup, infectious erythema, rabies, warts, West Nile fever, chicken-pox, cytomegalovirus (CMV), bacterial infectious diseases, such as abortion (infectious, septic), prostatitis (prostate inflammation), anthrax, appendicitis (inflammation of the caecum), borreliosis, botulism, Campylobacter, Chlamydia trachomatis (inflammation of the urethra, conjunctiva), cholera, diphtheria, donavonosis, epiglottitis, louse-borne typhus, typhoid fever, gas gangrene, gonorrhoea, hare plague, Helicobacter pylori, whooping-cough, climatic bubo, osteomyelitis, legionnaires' disease, leprosy, listeriosis, pneumonia, meningitis, bacterial meningitis, anthrax, inflammation of the middle ear, Mycoplasma hominis, neonatal sepsis (chorioamnionitis), noma, paratyphoid fever, plague, Reiter's syndrome, Rocky Mountain spotted fever, Salmonella paratyphoid fever, Salmonella typhoid fever, scarlet fever, syphilis, tetanus, gonorrhoea, tsutsugamushi fever, tuberculosis, typhus, vaginitis (colpitis), soft chancre, and infectious diseases caused by parasites, protozoa or fungi, such as amoebic dysentery, bilharziosis, Chagas' disease, Echinococcus, fish tapeworm, ichthyotoxism (ciguatera), fox tapeworm, mycosis pedis, dog tapeworm, candiosis, ptyriasis, the itch (scabies), cutaneous leishmaniasis, lamblian dysentery (giadiasis), lice, malaria, onchocercosis (river blindness), fungal diseases, beef tapeworm, schistosomiasis, sleeping sickness, pork tapeworm, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral leishmaniasis, nappy dermatitis, or infections caused by

the dwarf tapeworm.

33. Use according to one of items 29 or 30, wherein the cardiovascular diseases are chosen from the group consisting of coronary heart disease, arteriosclerosis, apoplexy and hypertension, and neuronal diseases chosen from Alzheimer's disease, amyotrophic lateral sclerosis, dystonia, epilepsy, multiple sclerosis and Parkinson's disease.

34. Use according to one of items 29 or 30, wherein the autoimmune diseases are chosen from the group consisting of autoimmune type I diseases or autoimmune type II diseases or autoimmune type III diseases or autoimmune type IV diseases, such as, for example, multiple sclerosis (MS), rheumatoid arthritis, diabetes, diabetes type I (diabetes mellitus), systemic lupus erythematosus (SLE), chronic polyarthritis, Basedow's disease, autoimmune forms of chronic hepatitis, colitis ulcerosa, allergy type I diseases, allergy type II diseases, allergy type III diseases, allergy type IV diseases, fibromyalgia, hair loss, Bechterew's disease, Crohn's disease, myasthenia gravis, neurodermatitis, polymyalgia rheumatica, progressive systemic sclerosis (PSS), psoriasis, Reiter's syndrome, rheumatic arthritis, psoriasis or vasculitis.

35. *In vitro* transcription method for the preparation of an antibody-coding, optionally modified RNA, comprising the following steps:

a) provision of a nucleic acid which codes for an antibody, as defined in items 4 to 6;
b) addition of the nucleic acid to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, a nucleic acid mix comprising one or more modified nucleotides in exchange for one or more of the naturally occurring nucleotides A, G, C or U, and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged, or optionally only naturally occurring nucleotides and optionally an RNase inhibitor;
c) incubation of the nucleic acid in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give an antibody-coding, optionally modified RNA according to items 1 to 27;
d) optionally purification of the antibody-coding, optionally modified RNA and removal of the non-incorporated nucleotides from the *in vitro* transcription medium.

36. *In vitro* transcription and translation method for expression of an antibody, comprising the following steps:

a) provision of a nucleic acid which codes for an antibody, as defined in items 4 to 6;
b) addition of the nucleic acid to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, a nucleic acid mix comprising one or more modified nucleotides in exchange for one or more of the naturally occurring nucleotides A, G, C or U, and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged, or optionally only naturally occurring nucleotides and optionally an RNase inhibitor;
c) incubation of the nucleic acid in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give an antibody-coding, optionally modified RNA according to items 1 to 27;
d) optionally purification of the antibody-coding, optionally modified RNA and removal of the non-incorporated nucleotides from the *in vitro* transcription medium,
e) addition of the optionally modified RNA obtained in step c) (and optionally in step d) to an *in vitro* translation medium;
f) incubation of the optionally modified RNA in the *in vitro* translation medium and *in vitro* translation of the antibody coded by the optionally modified RNA;
g) optionally purification of the antibody translated in step f).

37. *In vitro* transcription and translation method for expression of an antibody in a host cell, comprising the following steps:

a) provision of a nucleic acid which codes for an antibody, as defined in items 4 to 6;
b) addition of the nucleic acid to an *in vitro* transcription medium comprising an RNA polymerase, a suitable buffer, one or more modified nucleotides in exchange for one or more of the naturally occurring nucleotides A, G, C or U and optionally one or more naturally occurring nucleotides A, G, C or U, if not all the naturally occurring nucleotides A, G, C or U are to be exchanged, or only naturally occurring nucleotides and optionally an RNase inhibitor;
c) incubation of the nucleic acid in the *in vitro* transcription medium and *in vitro* transcription of the nucleic acid to give an antibody-coding, optionally modified RNA according to items 1 to 27;

d) optionally purification of the antibody-coding, optionally modified RNA according to the invention and removal of the non-incorporated nucleotides from the *in vitro* transcription medium,
e') transfection of the optionally modified RNA obtained in step c) (and optionally d)) into a host cell;
f) incubation of the optionally modified nucleic acid in the host cell and translation of the antibody coded by the optionally modified RNA in the host cell;
g') optionally isolation and/or purification of the antibody translated in step f).

SEQUENCE LISTING

<110> CureVac AG

<120> RNA-coded antibody

<130> CU01P048WOEPT2

<140>
<141>

<150> DE 102007001370.3-41
<151> 2007-01-09

<160> 53

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ccgcgccagg ggctggagtg gatcggcgcc atctaccccg ggaacggcga caccagctac 180

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<220>
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agcgtgaccg aacaggatag caaagatagc acctatagcc tgagcagcac cctgaccctg 540

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agcagcccgg tgaccaaaag ctttaaccgc taa 633

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<213> Artificial

<220>
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<213> Artificial

<220>
<223> Antibody Rituximab - modified RNA for the light and heavy chains in the total construct

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<213> Artificial

<220>
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<212> DNA
<213> Artificial

<220>
<223> Antibody Cetuximab (= Erbitux) - GC-optimized heavy chain

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<213> Artificial

<220>
<223> Antibody Cetuximab (= Erbitux) - total construct with GC-optimized heavy and light chains

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ctggtgaagg ggttctaccc gtcggacatc gccgtggagt gggagagcaa cggccagccc 1260

gagaacaact acaagaccac gcccccggtc ctggacagcg acggcagctt cttcctctac 1320

agcaagctga ccgtggacaa gagccgctgg cagcagggga acgtgttctc gtgcagcgtc 1380

atgcacgagg ccctgcacaa ccactacacc cagaagagcc tcagcctgag ccccggcaag 1440

catcatcatc atcatcattg accagatctt tctgacattt ctgacatttc tgacatttct 1500

gacatttctg acatttctga catttctgac atttctgaca tttctgacat ttctgacata 1560

tgcataccat ggccgtgatg gcgccgcgga ccctggtcct cctgctgagc ggcgccctcg 1620

ccctgacgca gacctgggcc ggggacatcc tgctcaccca gagcccggtg atcctgtcgg 1680

tcagccccgg cgagcgggtg agcttcagct gccgcgccag ccagtcgatc gggacgaaca 1740

tccactggta ccagcagcgg accaacggca gcccccgcct gctcatcaag tacgcgagcg 1800

agagcatcag cgggatcccc tcgcggttca gcggcagcgg gagcggcacc gacttcaccc 1860

tgagcatcaa cagcgtggag tcggaggaca tcgccgacta ctactgccag cagaacaaca 1920

actggccgac gaccttcggc gccgggacca agctggagct caagcgcacc gtcgccgcgc 1980

ccagcgtgtt catcttcccg cccagcgacg agcagctgaa gagcggcacg gccagcgtgg 2040

tctgcctgct caacaacttc taccccgggg aggccaaggt gcagtggaag gtggacaacg 2100

ccctgcagtc ggggaacagc caggagagcg tcaccgagca ggacagcaag gacagcacct 2160

acagcctgtc gagcaccctc acgctgagca aggccgacta cgagaagcac aaggtgtacg 2220

cgtgcgaggt gacccaccag ggcctgagca gccccgtcac caagtcgttc aaccgcggcg 2280

cctgaccact agt 2293

<210> 11
<211> 1356
<212> DNA
<213> Artificial

<220>
<223> Antibody Trastuzumab (= Herceptin) - wildtype, heavy chain

<400> 11
gaagtgcagc tggtggaaag cggcggcggc ctggtgcagc cgggcggcag cctgcgcctg 60

agctgcgcgg cgagcggctt taacattaaa gatacctata ttcattgggt gcgccaggcg 120

ccgggcaaag gcctggaatg ggtggcgcgc atttatccga ccaacggcta tacccgctat 180

gcggatagcg tgaaaggccg ctttaccatt agcgcggata ccagcaaaaa caccgcgtat 240

ctgcagatga acagcctgcg cgcggaagat accgcggtgt attattgcag ccgctggggc 300

ggcgatggct tttatgcgat ggattattgg ggccagggca ccctggtgac cgtgagcagc 360

gcgagcacca aaggcccgag cgtgtttccg ctggcgccga gcagcaaaag caccagcggc 420

ggcaccgcgg cgctgggctg cctggtgaaa gattattttc cggaaccggt gaccgtgagc 480

tggaacagcg gcgcgctgac cagcggcgtg cataccttc cggcggtgct gcagagcagc 540

ggcctgtata gcctgagcag cgtggtgacc gtgccgagca gcagcctggg cacccagacc 600

tatatttgca acgtgaacca taaaccgagc aacaccaaag tggataaaaa agtggaaccg 660

ccgaaaagct gcgataaaac ccatacctgc ccgccgtgcc cggcgccgga actgctgggc 720

ggcccgagcg tgtttctgtt tccgccgaaa ccgaaagata ccctgatgat tagccgcacc 780

ccggaagtga cctgcgtggt ggtggatgtg agccatgaag atccggaagt gaaatttaac 840

tggtatgtgg atggcgtgga agtgcataac gcgaaaacca aaccgcgcga agaacagtat 900

aacagcacct atcgcgtggt gagcgtgctg accgtgctgc atcaggattg gctgaacggc 960

aaagaatata aatgcaaagt gagcaacaaa gcgctgccgg cgccgattga aaaaaccatt 1020

agcaaagcga aaggccagcc gcgcgaaccg caggtgtata ccctgccgcc gagccgcgat 1080

gaactgacca aaaaccaggt gagcctgacc tgcctggtga aaggctttta tccgagcgat 1140

attgcggtgg aatgggaaag caacggccag ccggaaaaca actataaaac caccccgccg 1200

gtgctggata gcgatggcag ctttttctg tatagcaaac tgaccgtgga taaaagccgc 1260

tggcagcagg gcaacgtgtt tagctgcagc gtgatgcatg aagcgctgca taaccattat 1320

acccagaaaa gcctgagcct gagcccgggc aaataa 1356

<210> 12
<211> 1356
<212> DNA
<213> Artificial

<220>
<223> Antibody Trastuzumab (= Herceptin) - GC-optimized heavy chain

<400> 12
gaggtgcagc tggtcgagag cggcgggggc ctcgtgcagc cgggcgggtc gctgcggctg 60

agctgcgccg cgagcgggtt caacatcaag gacacctaca tccactgggt gcgccaggcc 120

cccggcaagg gcctcgagtg ggtcgcccgg atctacccca cgaacgggta cacccgctac 180

gccgacagcg tgaagggccg gttcaccatc agcgcggaca cctcgaagaa cacggcctac 240

ctgcagatga acagcctgcg cgccgaggac accgccgtgt actactgcag ccggtggggc 300

ggcgacgggt tctacgccat ggactactgg gggcagggca ccctcgtcac cgtgagcagc 360

gcgtcgacga aggggcccag cgtgttcccg ctggccccca gcagcaagag caccagcggc 420

gggaccgccg ccctgggctg cctcgtcaag gactacttcc ccgagcccgt gaccgtgtcg 480

tggaacagcg gcgcgctgac gagcggggtc cacaccttcc cggccgtgct gcagagcagc 540

ggcctctact cgctgagcag cgtggtcacc gtgcccagca gcagcctggg gacccagacg 600

tacatctgca acgtgaacca caagccctcg aacaccaagg tcgacaagaa ggtggagccc 660

ccgaagagct gcgacaagac ccacacctgc ccgccctgcc ccgcccccga gctcctgggc 720

gggcccagcg tgttcctgtt cccgcccaag cccaaggaca cgctcatgat cagccgcacc 780

cccgaggtca cctgcgtggt ggtcgacgtg agccacgagg accccgaggt gaagttcaac 840

tggtacgtcg acggcgtgga ggtgcacaac gccaagacca agccgcggga ggagcagtac 900

aactcgacgt accgcgtcgt gagcgtgctg accgtcctgc accaggactg gctcaacggc 960

aaggagtaca agtgcaaggt gagcaacaag gccctgcccg cgcccatcga gaagaccatc 1020

agcaaggcca aggggcagcc ccgggagccg caggtgtaca ccctgccccc cagccgcgac 1080

gagctcacga agaaccaggt cagcctgacc tgcctggtga agggcttcta cccctcggac 1140

atcgccgtgg agtgggagag caacgggcag ccggagaaca actacaagac caccccgccc 1200

gtcctcgaca gcgacggcag cttcttcctg tacagcaagc tgacggtgga caagtcgcgg 1260

tggcagcagg gcaacgtgtt cagctgcagc gtcatgcacg aggccctcca caaccactac 1320

acccagaaga gcctgagcct gagccccggg aagtga 1356

<210> 13
<211> 645
<212> DNA
<213> Artificial

<220>
<223> Antibody Trastuzumab (= Herceptin) - wildtype, light chain

<400> 13
gatattcaga tgacccagag cccgagcagc ctgagcgcga gcgtgggcga tcgcgtgacc 60

attacctgcc gcgcgagcca ggatgtgaac accgcggtgg cgtggtatca gcagaaaccg 120

ggcaaagcgc cgaaactgct gatttatagc gcgagctttc tgtatagcgg cgtgccgagc 180

cgctttagcg gcagccgcag cggcaccgat tttaccctga ccattagcag cctgcagccg 240

gaagattttg cgacctatta ttgccagcag cattatacca ccccgccgac ctttggccag 300

ggcaccaaag tggaaattaa acgcaccgtg gcggcgccga gcgtgtttat ttttccgccg 360

agcgatgaac agctgaaaag cggcaccgcg agcgtggtgt gcctgctgaa caacttttat 420

ccgcgcgaag cgaaagtgca gtggaaagtg gataacgcgc tgcagagcgg caacagccag 480

gaaagcgtga ccgaacagga tagcaaagat agcacctata gcctgagcag caccctgacc 540

ctgagcaaag cggattatga aaaacataaa gtgtatgcgt gcgaagtgac ccatcagggc 600

ctgagcagcc cggtgaccaa aagctttaac cgcggcgaat gctaa 645

<210> 14

<211> 645
<212> DNA
<213> Artificial

<220>
<223> Antibody Trastuzumab (= Herceptin) - GC-optimized light chain

<400> 14
gacatccaga tgacccagag cccgtcgagc ctgagcgcca gcgtgggcga ccgggtcacg 60

atcacctgcc gcgcgagcca ggacgtgaac accgccgtgg cctggtacca gcagaagccc 120

gggaaggccc ccaagctcct gatctactcg gcgagcttcc tgtacagcgg cgtccccagc 180

cggttcagcg ggtcgcgcag cggcaccgac ttcacgctca ccatcagcag cctgcagccg 240

gaggacttcg ccacctacta ctgccagcag cactacacca cgccccccac cttcgggcag 300

ggcaccaagg tggagatcaa gcggaccgtg gccgccccca gcgtcttcat cttcccgccc 360

agcgacgagc agctgaagtc gggcacggcc agcgtggtgt gcctcctgaa caacttctac 420

ccccgcgagg cgaaggtcca gtggaaggtg gacaacgccc tgcagagcgg gaacagccag 480

gagagcgtga ccgagcagga ctcgaaggac agcacctaca gcctcagcag caccctgacg 540

ctgagcaagg ccgactacga gaagcacaag gtctacgcct gcgaggtgac ccaccagggg 600

ctctcgagcc ccgtgaccaa gagcttcaac cggggcgagt gctga 645

<210> 15
<211> 2295
<212> DNA
<213> Artificial

<220>
<223> Antibody Trastuzumab (= Herceptin) - total construct with GC-optimized heavy und light chain

<400> 15
aagcttacca tggccgtgat ggcgccgcgg accctggtcc tcctgctgag cggcgccctc 60

gccctgacgc agacctgggc cggggaggtg cagctggtcg agagcggcgg gggcctcgtg 120

cagccgggcg ggtcgctgcg gctgagctgc gccgcgagcg ggttcaacat caaggacacc 180

tacatccact gggtgcgcca ggcccccggc aagggcctcg agtgggtcgc ccggatctac 240

cccacgaacg ggtacacccg ctacgccgac agcgtgaagg gccggttcac catcagcgcg 300

gacacctcga agaacacggc ctacctgcag atgaacagcc tgcgcgccga ggacaccgcc 360

gtgtactact gcagccggtg gggcggcgac gggttctacg ccatggacta ctgggggcag 420

ggcaccctcg tcaccgtgag cagcgcgtcg acgaaggggc ccagcgtgtt cccgctggcc 480

cccagcagca agagcaccag cggcgggacc gccgccctgg gctgcctcgt caaggactac 540

ttccccgagc ccgtgaccgt gtcgtggaac agcggcgcgc tgacgagcgg ggtccacacc 600

ttcccggccg tgctgcagag cagcggcctc tactcgctga gcagcgtggt caccgtgccc 660

agcagcagcc tggggaccca gacgtacatc tgcaacgtga accacaagcc ctcgaacacc 720

aaggtcgaca agaaggtgga gcccccgaag agctgcgaca agacccacac ctgcccgccc 780

tgccccgccc ccgagctcct gggcgggccc agcgtgttcc tgttcccgcc caagcccaag 840

gacacgctca tgatcagccg cacccccgag gtcacctgcg tggtggtcga cgtgagccac 900

gaggaccccg aggtgaagtt caactggtac gtcgacggcg tggaggtgca caacgccaag 960

accaagccgc gggaggagca gtacaactcg acgtaccgcg tcgtgagcgt gctgaccgtc 1020

ctgcaccagg actggctcaa cggcaaggag tacaagtgca aggtgagcaa caaggccctg 1080

cccgcgccca tcgagaagac catcagcaag gccaaggggc agccccggga gccgcaggtg 1140

tacaccctgc cccccagccg cgacgagctc acgaagaacc aggtcagcct gacctgcctg 1200

gtgaagggct tctacccctc ggacatcgcc gtggagtggg agagcaacgg gcagccggag 1260

aacaactaca agaccacccc gcccgtcctc gacagcgacg gcagcttctt cctgtacagc 1320

aagctgacgg tggacaagtc gcggtggcag cagggcaacg tgttcagctg cagcgtcatg 1380

cacgaggccc tccacaacca ctacacccag aagagcctga gcctgagccc cgggaagcat 1440

catcatcatc atcattgacc agatctttct gacatttctg acatttctga catttctgac 1500

atttctgaca tttctgacat ttctgacatt tctgacattt ctgacatttc tgacatatgc 1560

ataccatggc cgtgatggcg ccgcggaccc tggtcctcct gctgagcggc gccctcgccc 1620

tgacgcagac ctgggccggg gacatccaga tgacccagag cccgtcgagc ctgagcgcca 1680

gcgtgggcga ccgggtcacg atcacctgcc gcgcgagcca ggacgtgaac accgccgtgg 1740

cctggtacca gcagaagccc gggaaggccc ccaagctcct gatctactcg gcgagcttcc 1800

tgtacagcgg cgtccccagc cggttcagcg ggtcgcgcag cggcaccgac ttcacgctca 1860

ccatcagcag cctgcagccg gaggacttcg ccacctacta ctgccagcag cactacacca 1920

cgccccccac cttcgggcag ggcaccaagg tggagatcaa gcggaccgtg gccgccccca 1980

gcgtcttcat cttcccgccc agcgacgagc agctgaagtc gggcacggcc agcgtggtgt 2040

gcctcctgaa caacttctac ccccgcgagg cgaaggtcca gtggaaggtg gacaacgccc 2100

tgcagagcgg gaacagccag gagagcgtga ccgagcagga ctcgaaggac agcacctaca 2160

gcctcagcag caccctgacg ctgagcaagg ccgactacga gaagcacaag gtctacgcct 2220

gcgaggtgac ccaccagggg ctctcgagcc ccgtgaccaa gagcttcaac cggggcgagt 2280

gctgatgacc actag 2295

<210> 16
<211> 13
<212> RNA
<213> Artificial

<220>
<223> Description of sequence: Kozak-sequence

<400> 16
gccgccacca ugg 13

<210> 17
<211> 15
<212> RNA
<213> Artificial

<220>
<223> Description of sequence: generic stabilizing sequence

<220>
<221> variation
<222> (1)..(1)
<223> /replace="cytosine"
/replace="uracile"

<220>
<221> variation
<222> (5)..(5)
<223> /replace="cytosine"
/replace="uracile"
/replace="guanosine"
/replace="adenosine", or any other nucleic acid

<220>
<221> repeat_unit
<222> (5)..(5)
<223> x = any number

<220>
<221> variation
<222> (9)..(9)
<223> /replace="uracile"
/replace="adonosine"

<220>
<221> repeat_unit
<222> (10)..(10)
<223> x = any number

<220>
<221> variation
<222> (10)..(10)
<223> /replace="pyrimidine"

<220>
<221> variation
<222> (13)..(13)
<223> /replace="cytosine"
/replace="uracile"

<400> 17
nccancccnn ucncc 15

<210> 18
<211> 4
<212> PRT

<213> Artificial

<220>
<223> localization sequence for the endoplasmic reticulum

<400> 18

Lys Asp Glu Leu
1

<210> 19
<211> 4
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the endoplasmic reticulum

<400> 19

Asp Asp Glu Leu
1

<210> 20
<211> 4
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the endoplasmic reticulum

<400> 20

Asp Glu Glu Leu
1

<210> 21
<211> 4
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the endoplasmic reticulum

<400> 21

Gln Glu Asp Leu
1

<210> 22
<211> 4
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the endoplasmic reticulum

<400> 22

Arg Asp Glu Leu
1

<210> 23
<211> 7
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the nucleus

<400> 23

Pro Lys Lys Lys Arg Lys Val
1 5

<210> 24
<211> 7
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the nucleus

<400> 24

Pro Gln Lys Lys Ile Lys Ser
1 5

<210> 25
<211> 5
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the nucleus

<400> 25

Gln Pro Lys Lys Pro
1 5

<210> 26
<211> 4
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the nucleus

<400> 26

Arg Lys Lys Arg
1

<210> 27
<211> 12
<212> PRT

<213> Artificial

<220>
<223> localization sequence for the nuclear region

<400> 27

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His Gln
1 5 10

<210> 28
<211> 16
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the nuclear region

<400> 28

Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg
1 5 10 15

<210> 29
<211> 19
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the nuclear region

<400> 29

Met Pro Leu Thr Arg Arg Arg Pro Ala Ala Ser Gln Ala Leu Ala Pro
1 5 10 15

Pro Thr Pro

<210> 30
<211> 15
<212> PRT
<213> Artificial

<220>
<223> localization sequence for the endosomal compartiment

<400> 30

Met Asp Asp Gln Arg Asp Leu Ile Ser Asn Asn Glu Gln Leu Pro
1 5 10 15

<210> 31
<211> 32
<212> PRT
<213> Artificial

<220>

<223> Myristoylation sequence, likalizing the antibody to the mitochondrial matrix

<220>
<221> misc_feature
<222> (7)..(8)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (32)..(32)
<223> Xaa can be any naturally occurring amino acid

<400> 31

Met Leu Phe Asn Leu Arg Xaa Xaa Leu Asn Asn Ala Ala Phe Arg His
1 5 10 15

Gly His Asn Phe Met Val Arg Asn Phe Arg Cys Gly Gln Pro Leu Xaa
20 25 30

<210> 32
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 32

Gly Cys Val Cys Ser Ser Asn Pro
1 5

<210> 33
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 33

Gly Gln Thr Val Thr Thr Pro Leu
1 5

<210> 34
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 34

Gly Gln Glu Leu Ser Gln His Glu
1 5

<210> 35
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 35

Gly Asn Ser Pro Ser Tyr Asn Pro
1 5

<210> 36
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 36

Gly Val Ser Gly Ser Lys Gly Gln
1 5

<210> 37
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 37

Gly Gln Thr Ile Thr Thr Pro Leu
1 5

<210> 38
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 38

Gly Gln Thr Leu Thr Thr Pro Leu
1 5

<210> 39
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 39

Gly Gln Ile Phe Ser Arg Ser Ala
1 5

<210> 40
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 40

Gly Gln Ile His Gly Leu Ser Pro
1 5

<210> 41
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 41

Gly Ala Arg Ala Ser Val Leu Ser
1 5

<210> 42
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane

<400> 42

Gly Cys Thr Leu Ser Ala Glu Glu
1 5

<210> 43
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the endoplasmic reticulum

<400> 43

Gly Gln Asn Leu Ser Thr Ser Asn
1 5

<210> 44
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the nucleus

<400> 44

Gly Ala Ala Leu Thr Ile Leu Val
1 5

<210> 45
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the nucleus

<400> 45

Gly Ala Ala Leu Thr Leu Leu Gly
1 5

<210> 46
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the nucleus endoplasmic reticulum and the cytoplasma

<400> 46

Gly Ala Gln Val Ser Ser Gln Lys
1 5

<210> 47
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the nucleus endoplasmic reticulum and the cytoplasma

<400> 47

Gly Ala Gln Leu Ser Arg Asn Thr
1 5

<210> 48
<211> 8
<212> PRT

<213> Artificial

<220>
<223> sequence for localization of antibodies to the golgi apparatus, the nucleus, the cytoplasma and the cytoskeleton

<400> 48

Gly Asn Ala Ala Ala Ala Lys Lys
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the cytoplasma and the cytoskeleton

<400> 49

Gly Asn Glu Ala Ser Tyr Pro Leu
1 5

<210> 50
<211> 8
<212> PRT
<213> Artificial

<220>
<223> sequence for localization of antibodies to the plasma membrane and the cytoskeleton

<400> 50

Gly Ser Ser Lys Ser Lys Pro Lys
1 5

<210> 51
<211> 2269
<212> DNA
<213> Artificial

<220>
<223> Alternative sequence of the construct of Fig. 12 (antibody rituximab), wherein the restriction sites have been modified as compared to SEQ ID No. 5 of Fig. 12.

<400> 51
aagcttacca tggccgtgat ggcgccgcgg accctggtcc tcctgctgag cggcgccctc 60

gccctgacgc agacctgggc cgggcaggcc tacctgcagc agagcggcgc ggagctcgtg 120

cggccggggg cctcggtcaa gatgagctgc aaggccagcg gctacacctt cacgagctac 180

aacatgcact gggtgaagca gaccccgcgc caggggctgg agtggatcgg cgccatctac 240

cccgggaacg gcgacaccag ctacaaccag aagttcaagg gcaaggcgac cctgacggtg 300

gacaagtcga gcagcaccgc ctacatgcag ctcagcagcc tgacctcgga ggacagcgcc 360

gtctacttct gcgcccgggt ggtgtactac agcaacagct actggtactt cgacgtctgg 420

gggaccggca cgaccgtgac cgtgagcggg cccagcgtct tccccctggc cccctcgagc 480

aagagcacca gcggcggcac ggcggccctc gggtgcctgg tgaaggacta cttccccgag 540

cccgtgaccg tcagctggaa ctcgggcgcc ctgaccagcg gggtgcacac cttcccggcc 600

gtgctccaga gcagcggcct gtacagcctg agctcggtcg tgacggtgcc cagcagcagc 660

ctcgggaccc agacctacat ctgcaacgtc aaccacaagc ccagcaacac caaggtggac 720

aagaaggcgg agcccaagtc gtgcgacaag acgcacacct gcccgccctg ccccgccccc 780

gagctgctgg gcggcccgag cgtgttcctc ttcccgccca agcccaagga caccctgatg 840

atcagccgca cccccgaggt cacgtgcgtg gtggtcgacg tgagccacga ggaccccgag 900

gtgaagttca actggtacgt cgacggggtg gaggtgcaca acgccaagac caagccccgg 960

gaggagcagt acaacagcac ctaccgcgtc gtgagcgtgc tgaccgtcct ccaccaggac 1020

tggctgaacg gcaaggagta caagtgcaag gtgtcgaaca aggccctgcc ggcccccatc 1080

gagaagacga tcagcaaggc gaaggggcag ccccgggagc cccaggtgta caccctcccg 1140

cccagccgcg acgagctgac caagaaccag gtcagcctga cctgcctcgt gaagggcttc 1200

taccccagcg acatcgccgt ggagtgggag tcgaacgggc agcccgagaa caactacaag 1260

acgaccccgc ccgtcctgga cagcgacggc agcttcttcc tgtacagcaa gctcaccgtg 1320

gacaagagcc ggtggcagca gggcaacgtg ttcagctgct cggtcatgca cgaggccctg 1380

cacaaccact acacccagaa gagcctgagc ctcagccccg ggaagcatca tcatcatcat 1440

cattgaccat gcatttctga catttctgac atttctgaca tttctgacat ttctgacatt 1500

tctgacattt ctgacatttc tgacatttct gacatttctg acatagatct accatggccg 1560

tgatggcgcc gcggaccctg gtcctcctgc tgagcggcgc cctcgccctg acgcagacct 1620

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agcccgggag cagccccaag ccgtggatct acgcccccag caacctggcc tcgggcgtgc 1800

ccgcgcgctt cagcgggagc ggcagcggga ccagctacag cctgaccatc tcgcgggtcg 1860

aggccgagga cgccgccacc tactactgcc agcagtggag cttcaacccg cccacgttcg 1920

gcgccggcac caagctcgag ctgaagcgca ccgtggcggc ccccagcgtg ttcatcttcc 1980

cgcccagcga cgagcagctg aagagcggga ccgcctcggt cgtgtgcctc ctgaacaact 2040

tctacccccg ggaggccaag gtgcagtgga aggtcgacaa cgcgctgcag agcggcaaca 2100

gccaggagag cgtgacggag caggacagca aggacagcac ctactcgctc agcagcaccc 2160

tgaccctgag caaggccgac tacgagaagc acaaggtgta cgcctgcgag gtcacgcacc 2220

aggggctcag ctcgcccgtg accaagagct tcaaccgctg accactagt 2269

<210> 52
<211> 2293
<212> DNA
<213> Artificial

<220>
<223> Alternative sequence of the construct of Fig. 17 (antibody cetuximab), wherein the restriction sites have been modified as compared to SEQ ID No. 10 of Fig. 17.

<400> 52
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gccctgacgc agacctgggc cgggcaggtg cagctgaagc agagcggccc ggggctcgtc 120

cagccctcgc agagcctgag catcacctgc acggtgagcg gcttcagcct gaccaactac 180

ggggtgcact gggtccggca gtcgcccggc aaggggctcg agtggctggg cgtgatctgg 240

agcggcggga acaccgacta caacaccccc ttcacgagcc gcctgagcat caacaaggac 300

aacagcaagt cgcaggtgtt cttcaagatg aacagcctcc agagcaacga caccgccatc 360

tactactgcg cgcgggccct gacctactac gactacgagt tcgcctactg gggccagggg 420

accctggtca cggtgagcgc cgcgagcacc aagggcccga gcgtgttccc cctcgccccc 480

tcgagcaaga gcaccagcgg cgggaccgcc gccctgggct gcctggtcaa ggactacttc 540

cccgagccgg tgacggtgag ctggaactcg ggggccctca ccagcggcgt ccacaccttc 600

cccgcggtgc tgcagagcag cgggctgtac agcctcagct cggtggtcac cgtgcccagc 660

agcagcctgg gcacgcagac ctacatctgc aacgtgaacc acaagcccag caacaccaag 720

gtcgacaagc gcgtggagcc gaagtcgccc aagagctgcg acaagaccca cacgtgcccg 780

ccctgccccg cccccgagct gctcggcggg cccagcgtgt tcctgttccc gcccaagccc 840

aaggacaccc tgatgatcag ccggaccccc gaggtcacct gcgtggtggt cgacgtgagc 900

cacgaggacc cggaggtgaa gttcaactgg tacgtcgacg gcgtggaggt gcacaacgcc 960

aagacgaagc cccgcgagga gcagtacaac agcacctacc gggtcgtgtc ggtgctcacc 1020

gtcctgcacc aggactggct gaacgggaag gagtacaagt gcaaggtgag caacaaggcc 1080

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gtgtacacgc tgcccccccag ccgggacgag ctgaccaaga accaggtcag cctcacctgc 1200

ctggtgaagg ggttctaccc gtcggacatc gccgtggagt gggagagcaa cggccagccc 1260

gagaacaact acaagaccac gcccccggtc ctggacagcg acggcagctt cttcctctac 1320

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gatctaccat ggccgtgatg gcgccgcgga ccctggtcct cctgctgagc ggcgccctcg 1620

ccctgacgca gacctgggcc ggggacatcc tgctcaccca gagcccggtg atcctgtcgg 1680

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ccagcgtgtt catcttcccg cccagcgacg agcagctgaa gagcggcacg gccagcgtgg 2040

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ccctgcagtc ggggaacagc caggagagcg tcaccgagca ggacagcaag gacagcacct 2160

acagcctgtc gagcaccctc acgctgagca aggccgacta cgagaagcac aaggtgtacg 2220

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cctgaccact agt 2293

<210> 53
<211> 2295
<212> DNA
<213> Artificial

<220>
<223> Alternative sequence of the construct of Fig. 22 (antibody trastuzumab), wherein the restriction sites have been modified as compared to SEQ ID No. 15 of Fig. 22.

<400> 53
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cagccgggcg ggtcgctgcg gctgagctgc gccgcgagcg ggttcaacat caaggacacc 180

tacatccact gggtgcgcca ggcccccggc aagggcctcg agtgggtcgc ccggatctac 240

cccacgaacg ggtacacccg ctacgccgac agcgtgaagg gccggttcac catcagcgcg 300

gacacctcga agaacacggc ctacctgcag atgaacagcc tgcgcgccga ggacaccgcc 360

gtgtactact gcagccggtg gggcggcgac gggttctacg ccatggacta ctgggggcag 420

ggcaccctcg tcaccgtgag cagcgcgtcg acgaaggggc ccagcgtgtt cccgctggcc 480

cccagcagca agagcaccag cggcgggacc gccgccctgg gctgcctcgt caaggactac 540

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agcagcagcc tggggaccca gacgtacatc tgcaacgtga accacaagcc ctcgaacacc 720

aaggtcgaca agaaggtgga gcccccgaag agctgcgaca agacccacac ctgcccgccc 780

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gtgaagggct tctacccctc ggacatcgcc gtggagtggg agagcaacgg gcagccggag 1260

aacaactaca agaccacccc gcccgtcctc gacagcgacg gcagcttctt cctgtacagc 1320

aagctgacgg tggacaagtc gcggtggcag cagggcaacg tgttcagctg cagcgtcatg 1380

cacgaggccc tccacaacca ctacacccag aagagcctga gcctgagccc cgggaagcat 1440

catcatcatc atcattgacc atgcatttct gacatttctg acatttctga catttctgac 1500

atttctgaca tttctgacat ttctgacatt tctgacattt ctgacatttc tgacatagat 1560

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gcgtgggcga ccgggtcacg atcacctgcc gcgcgagcca ggacgtgaac accgccgtgg 1740

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gcgaggtgac ccaccagggg ctctcgagcc ccgtgaccaa gagcttcaac cggggcgagt 2280

gctgatgacc actag 2295

Claims

1. RNA for intracellular expression of an antibody, wherein the RNA contains at least one coding region, wherein the at least one coding region codes for at least one antibody or codes for at least one heavy chain of an antibody or codes for at least one light chain of an antibody, wherein the RNA forms a complex with suitable carriers and wherein the carriers are liposomes.

2. RNA according to claim 1, wherein the RNA is single-stranded or double-stranded, linear or circular.

3. RNA according to claim 1 or 2, wherein the RNA is an mRNA.

4. RNA according to claim 1 to 3, wherein the antibody coded is chosen from monoclonal and polyclonal antibodies, chimeric antibodies, human antibodies, humanized antibodies, bispecific antibodies, intrabodies and fragments of these antibodies.

5. RNA according to claim 1 to 4, wherein the coded antibody fragments are chosen from Fab, Fab', $F(ab')_2$, Fc, Facb, pFc', Fd, and Fv or scFv fragments of these antibodies.

6. RNA according to claim 1 to 5, wherein the coded antibodies or antibody fragments specifically recognize and bind tumour-specific surface antigens chosen from (TSSA), 5T4, $\alpha 5\beta 1$-integrin, 707-AP, AFP, ART-4, B7H4, BAGE, β-catenin/m, Bcr-abl, MN/C IX-antigen, CA125, CAMEL, CAP-1, CASP-8, β-catenin/m, CD4, CD19, CD20, CD22, CD25, CDC27/m, CD 30, CD33, CD52, CD56, CD80, CDK4/m, CEA, CT, Cyp-B, DAM, EGFR, ErbB3, ELF2M, EMMPRIN, EpCam, ETV6-AML1, G250, GAGE, GnT-V, Gp100, HAGE, HER-2/neu, HLA-A*0201-R170I, HPV-E7, HSP70-2M, HAST-2, hTERT (or hTRT), iCE, IGF-1R, IL-2R, IL-5, KIAA0205, LAGE, LDLR/FUT, MAGE, MART-1/Melan-A, MART-2/Ski, MC1R, myosin/m, MUC1, MUM-1, -2, -3, NA88-A, PAP, proteinase-3, p190 minor bcr-abl, Pml/RARα, PRAME, PSA, PSM, PSMA, RAGE, RU1 or RU2, SAGE, SART-1 or SART-3, survivin, TEL/AML1, TGFβ, TPI/m, TRP-1, TRP-2, TRP-2/INT2, VEGF and WT1, NY-Eso-1 and NY-Eso-B.

7. RNA according to one of claims 1 to 6, wherein the RNA is modified.

8. RNA according to one of the preceding claims, wherein the modification is chosen from modifications of the nucleotide sequence compared with a precursor RNA sequence by introduction of non-native nucleotides and/or by covalent coupling of the RNA with another group.

9. RNA according to claim 8, wherein the RNA has a G/C content in the coding region of the base-modified RNA which is greater than the G/C content of the coding region of the native RNA sequence, the coded amino acid sequence being unchanged with respect to the wild-type or, respectively, the precursor RNA.

10. RNA according to claim 8 or 9, wherein the coding region of the modified RNA is modified compared with the coding region of the native RNA such that at least one codon of the native RNA which codes for a tRNA which is relatively rare in the cell is exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and which carries the same amino acid as the relatively rare tRNA.

11. RNA according to one of claims 8 to 10, wherein the RNA has a lipid modification.

12. RNA according to one of claims 8 to 11, wherein the RNA contains on at least one nucleotide of the RNA a modification of a nucleotide, wherein the nucleotides are chosen from 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), pseudouracil, 1-methyl-pseudouracil, queosine, β-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine.

13. RNA according to one of claims 8 to 12, wherein the RNA contains on at least one nucleotide of the RNA a modification

of a nucleotide, wherein the nucleotides are base-modified nucleotides chosen from the group consisting of 2-amino-6-chloropurine riboside 5'-triphosphate, 2-aminoadenosine 5'-triphosphate, 2-thiocytidine 5'-triphosphate, 2-thiouridine 5'-triphosphate, 4-thiouridine 5'-triphosphate, 5-aminoallylcytidine 5'-triphosphate, 5-aminoallyluridine 5'-triphosphate, 5-bromocytidine 5'-triphosphate, 5-bromouridine 5'-triphosphate, 5-iodocytidine 5'-triphosphate, 5-iodouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate, 5-methyluridine 5'-triphosphate, 6-azacytidine 5'-triphosphate, 6-azauridine 5'-triphosphate, 6-chloropurine riboside 5'-triphosphate, 7-deazaadenosine 5'-triphosphate, 7-deazaguanosine 5'-triphosphate, 8-azaadenosine 5'-triphosphate, 8-azidoadenosine 5'-triphosphate, benzimidazole riboside 5'-triphosphate, N1-methyladenosine 5'-triphosphate, N1-methylguanosine 5'-triphosphate, N6-methyladenosine 5'-triphosphate, 06-methylguanosine 5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate.

14. RNA according to claim 13, wherein the base-modified nucleotides are chosen from the group consisting of 5-methylcytidine 5'-triphosphate and pseudouridine 5'-triphosphate.

15. RNA according to one of the preceding claims, wherein the RNA additionally has a 5' cap structure chosen from the group consisting of m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G.

16. RNA according to one of the preceding claims, wherein the RNA additionally has a poly-A tail of from about 10 to 200 adenosine nucleotides, and/or wherein the RNA additionally has a poly-C tail of from about 10 to 200 cytosine nucleotides.

17. RNA according to one of the preceding claims, wherein the RNA additionally codes a tag for purification chosen from the group consisting of a hexahistidine tag (HIS tag, polyhistidine tag), a streptavidin tag (Strep tag), an SBP tag (streptavidin-binding tag) or a GST (glutathione S-transferase) tag, or codes for a tag for purification via an antibody epitope chosen from the group consisting of antibody-binding tags, a Myc tag, a Swa11 epitope, a FLAG tag or an HA tag.

18. RNA according to one of the preceding claims, wherein the RNA additionally codes a signal peptide and/or a localization sequence, in particular a secretion sequence.

19. Pharmaceutical composition comprising an RNA according to one of claims 1 to 18.

20. Combination of two RNAs according to any of claims 1 to 18 for intracellular expression of an antibody, wherein each of the first and the second RNA contains at least one coding region, wherein the at least one coding region of the first RNA codes for a heavy chain of an antibody and wherein the at least one coding region of the second RNA codes for a light chain of an antibody.

21. Use of an RNA sequence as defined in claims 1 to 18 for the preparation of a pharmaceutical composition for treatment of cancer diseases, cardiovascular diseases, infectious diseases or autoimmune diseases.

22. Use according to claim 21, wherein the pharmaceutical composition is in the form of a passive vaccine for treatment of tumour or infectious diseases.

23. Use according to one of claims 21 or 22, wherein the cancer diseases or tumour diseases are chosen from the group consisting of melanomas, malignant melanomas, colon carcinomas, lymphomas, sarcomas, blastomas, kidney carcinomas, gastrointestinal tumours, gliomas, prostate tumours, bladder cancer, rectal tumours, stomach cancer, oesophageal cancer, pancreatic cancer, liver cancer, mammary carcinomas (= breast cancer), uterine cancer, cervical cancer, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), hepatomas, diverse virus-induced tumours, such as e.g. papilloma virus-induced carcinomas (e.g. cervix carcinoma = cervical cancer), adenocarcinomas, herpes virus-induced tumours (e.g. Burkitt's lymphoma, EBV-induced B cell lymphoma), hepatitis B-induced tumours (hepatocell carcinomas), HTLV-1- and HTLV-2-induced lymphomas, acusticus neurinoma, lung carcinomas (= lung cancer = bronchial carcinoma), small cell lung carcinomas, throat cancer, anal carcinoma, glioblastoma, rectum carcinoma, astrocytoma, brain tumours, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, testicular cancer, thyroid carcinoma, Hodgkin's syndrome, meningeomas, Schneeberger's disease, pituitary tumour, mycosis fungoides, carcinoids, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, kidney cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumours, oligodendroglioma, vulval cancer, intestinal cancer, colon carcinoma, oesophageal carcinoma (= oesophageal cancer), wart

conditions, small intestine tumours, craniopharyngeomas, ovarian carcinoma, soft tissue tumours (sarcomas), ovarian cancer (= ovarian carcinoma), pancreatic carcinoma (= pancreatic cancer), endometrium carcinoma, liver metastases, penis cancer, tongue cancer, gallbladder cancer, leukaemia, plasmocytoma, lid tumour and prostate cancer (= prostate tumours) or wherein the infectious diseases are chosen from the group consisting of influenza, malaria, SARS, yellow fever, AIDS, Lyme borreliosis, leishmaniasis, anthrax, meningitis, viral infectious diseases, such as AIDS, condyloma acuminata, molluscum contagiosum, dengue fever, three-day fever, Ebola virus, colds, early summer meningoencephalitis (ESME), influenza, shingles, hepatitis, herpes simplex type I, herpes simplex type II, herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, measles, foot and mouth disease, mononucleosis, mumps, Norwalk virus infection, Pfeiffer's glandular fever, smallpox, polio (poliomyelitis), pseudocroup, infectious erythema, rabies, warts, West Nile fever, chicken-pox, cytomegalovirus (CMV), bacterial infectious diseases, such as abortion (infectious, septic), prostatitis (prostate inflammation), anthrax, appendicitis (inflammation of the caecum), borreliosis, botulism, Campylobacter, Chlamydia trachomatis (inflammation of the urethra, conjunctiva), cholera, diphtheria, donavonosis, epiglottitis, louse-borne typhus, typhoid fever, gas gangrene, gonorrhoea, hare plague, Helicobacter pylori, whooping-cough, climatic bubo, osteomyelitis, legionnaires' disease, leprosy, listeriosis, pneumonia, meningitis, bacterial meningitis, anthrax, inflammation of the middle ear, Mycoplasma hominis, neonatal sepsis (chorioamnionitis), noma, paratyphoid fever, plague, Reiter's syndrome, Rocky Mountain spotted fever, Salmonella paratyphoid fever, Salmonella typhoid fever, scarlet fever, syphilis, tetanus, gonorrhoea, tsutsugamushi fever, tuberculosis, typhus, vaginitis (colpitis), soft chancre, and infectious diseases caused by parasites, protozoa or fungi, such as amoebic dysentery, bilharziosis, Chagas' disease, Echinococcus, fish tapeworm, ichthyotoxism (ciguatera), fox tapeworm, mycosis pedis, dog tapeworm, candiosis, ptyriasis, the itch (scabies), cutaneous leishmaniasis, lamblian dysentery (giadiasis), lice, malaria, onchocercosis (river blindness), fungal diseases, beef tapeworm, schistosomiasis, sleeping sickness, pork tapeworm, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral leishmaniasis, nappy dermatitis, or infections caused by the dwarf tapeworm or wherein the cardiovascular diseases are chosen from the group consisting of coronary heart disease, arteriosclerosis, apoplexy and hypertension, or wherein the autoimmune diseases are chosen from the group consisting of autoimmune type I diseases or autoimmune type II diseases or autoimmune type III diseases or autoimmune type IV diseases, such as, for example, multiple sclerosis (MS), rheumatoid arthritis, diabetes, diabetes type I (diabetes mellitus), systemic lupus erythematosus (SLE), chronic polyarthritis, Basedow's disease, autoimmune forms of chronic hepatitis, colitis ulcerosa, allergy type I diseases, allergy type II diseases, allergy type III diseases, allergy type IV diseases, fibromyalgia, hair loss, Bechterew's disease, Crohn's disease, myasthenia gravis, neurodermatitis, polymyalgia rheumatica, progressive systemic sclerosis (PSS), psoriasis, Reiter's syndrome, rheumatic arthritis, psoriasis or vasculitis.

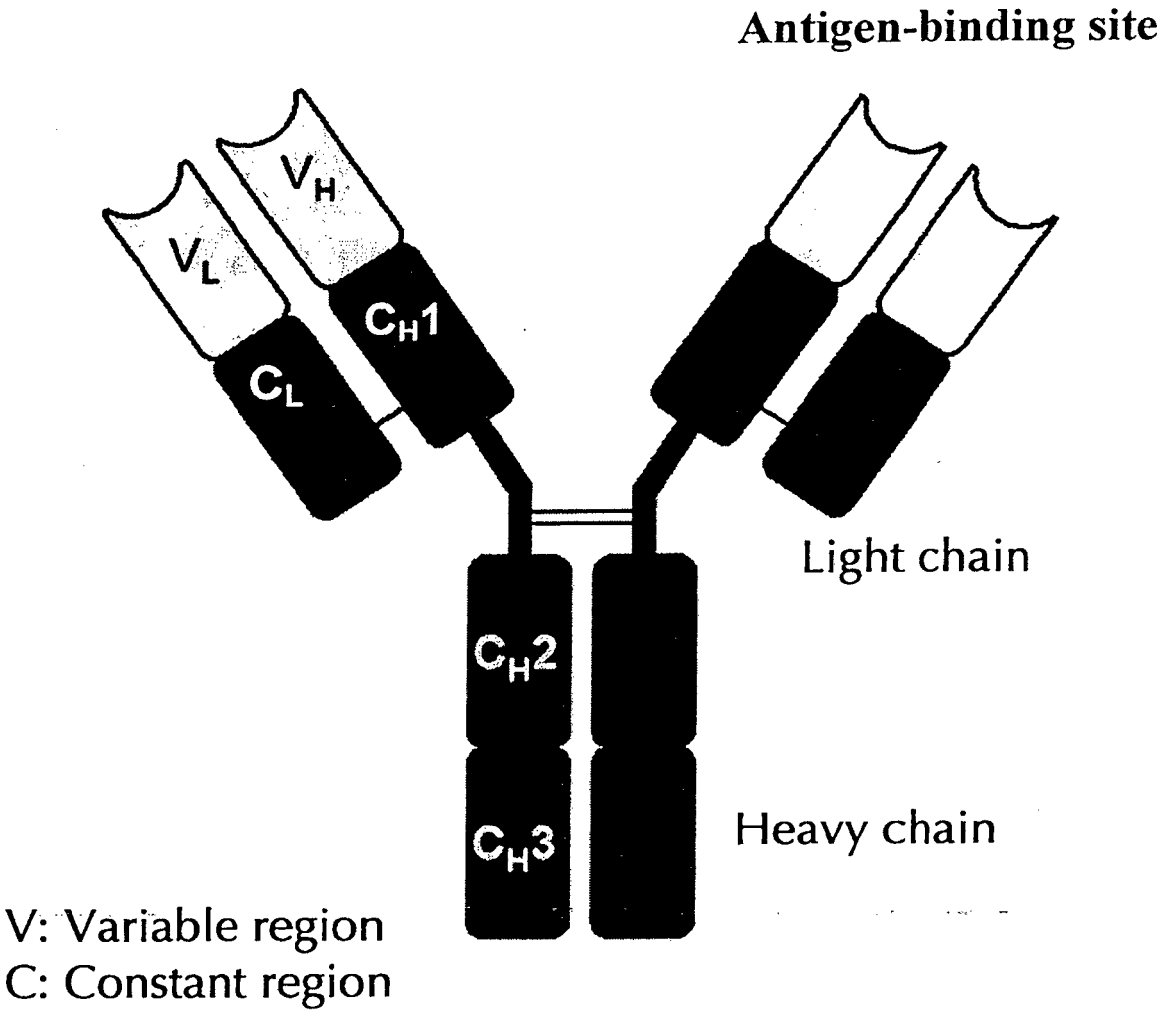
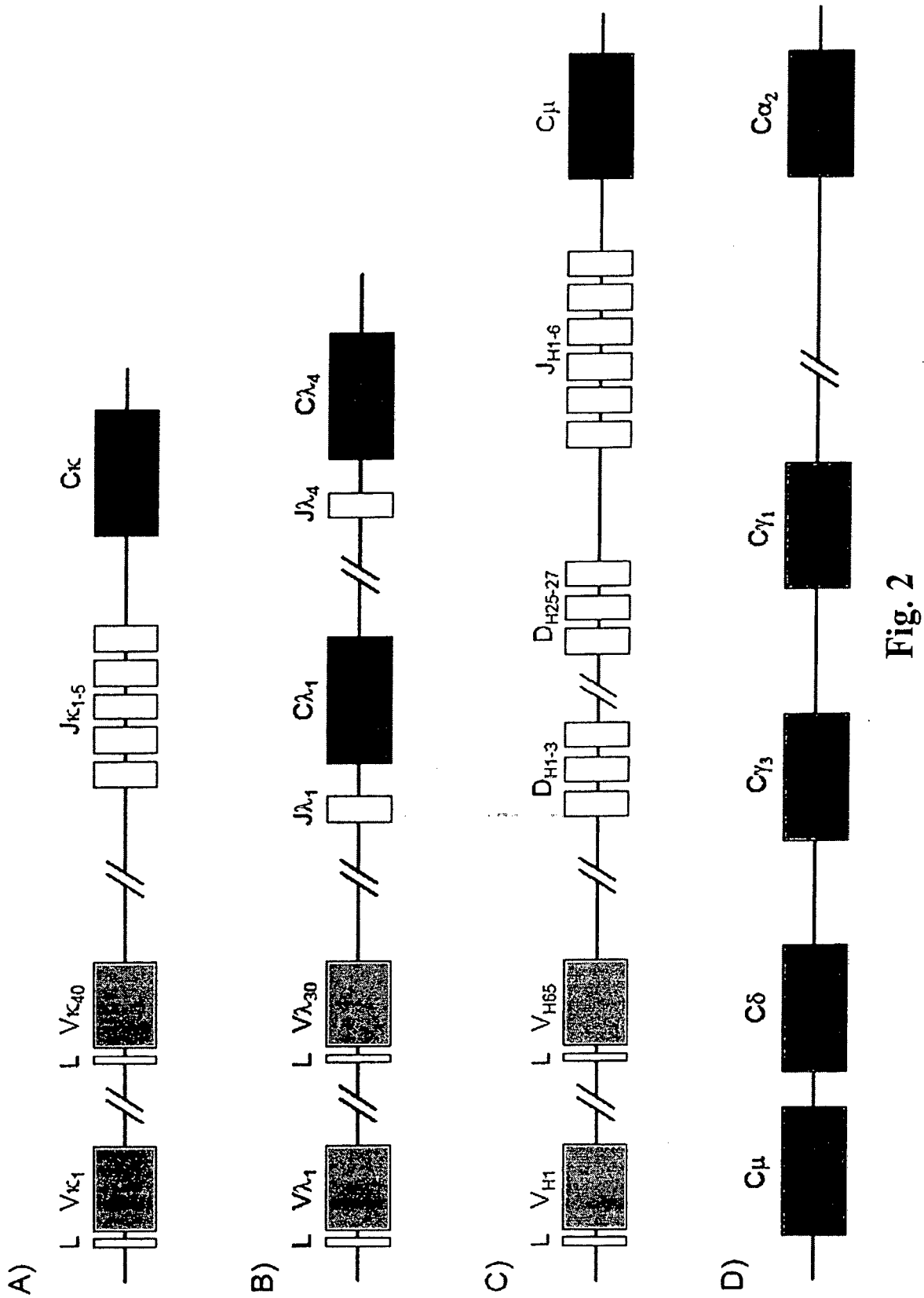

Antigen-binding site
V_H
V_L
C_H1
C_L
Light chain
C_H2
C_H3
Heavy chain
V: Variable region
C: Constant region

Fig. 1


A)
L
V_{K1}
L
V_{K40}
J_{K1-5}
C_K
B)
L
$V_{\lambda 1}$
L
$V_{\lambda 30}$
$J_{\lambda 1}$
$C_{\lambda 1}$
$J_{\lambda 4}$
$C_{\lambda 4}$
C)
L
V_{H1}
L
V_{H65}
D_{H1-3}
D_{H25-27}
J_{H1-6}
C_μ
D)
C_μ
C_δ
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$C_{\gamma 1}$
$C_{\alpha 2}$

Fig. 2

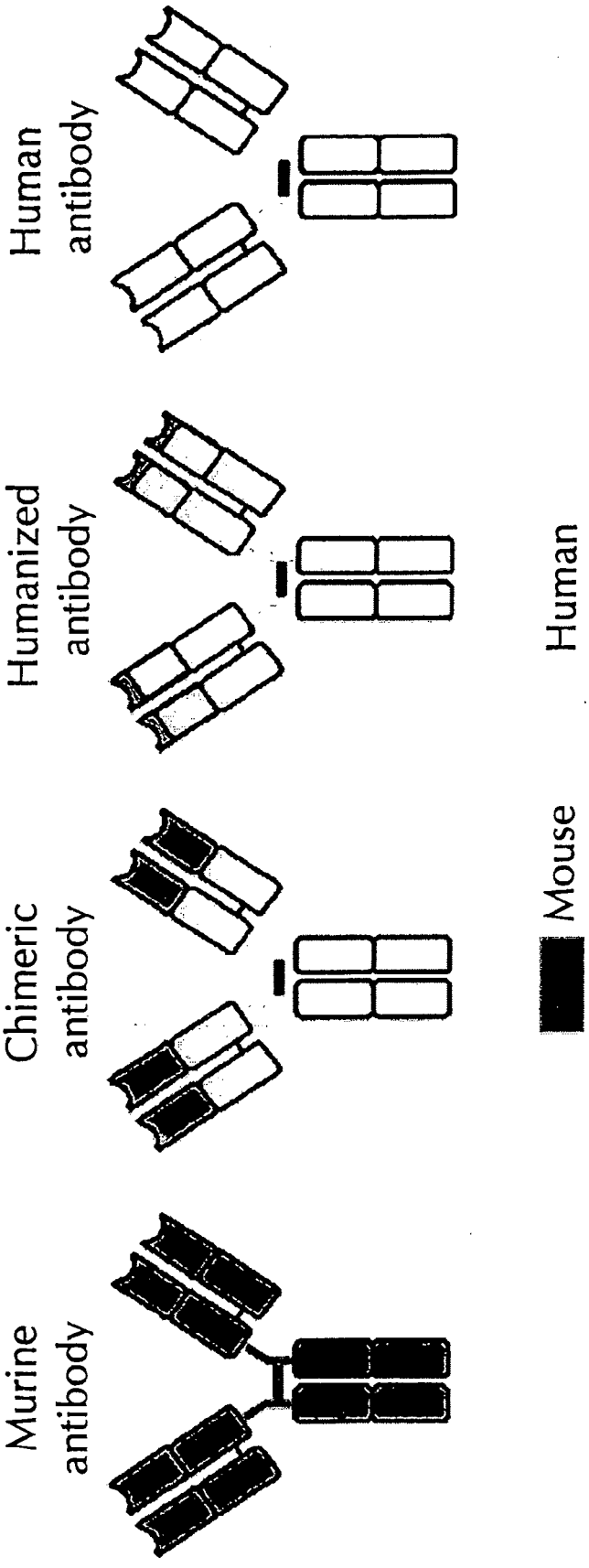
Murine antibody
Chimeric antibody
Humanized antibody
Human antibody
Mouse
Human

Fig. 3

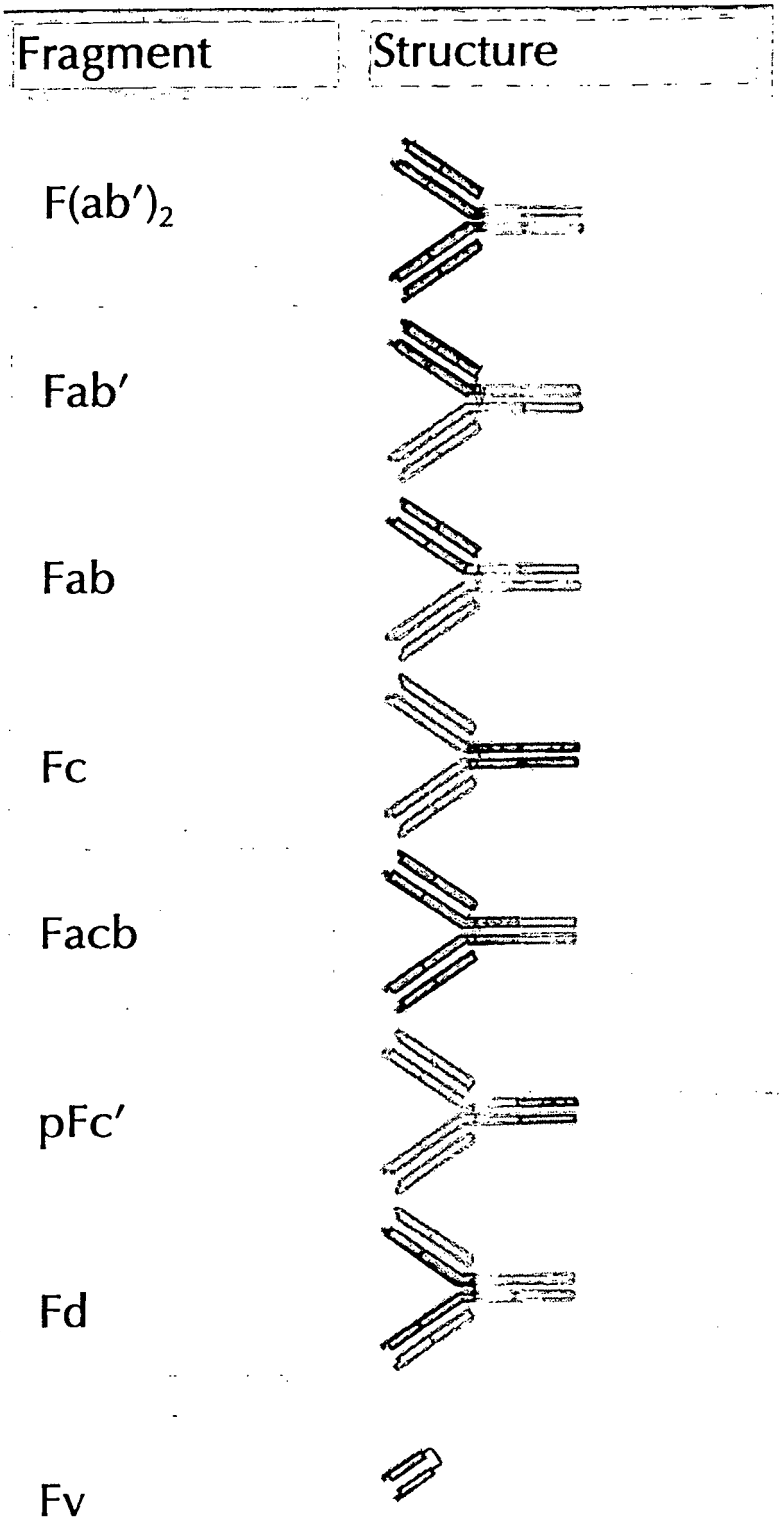

Fig. 4

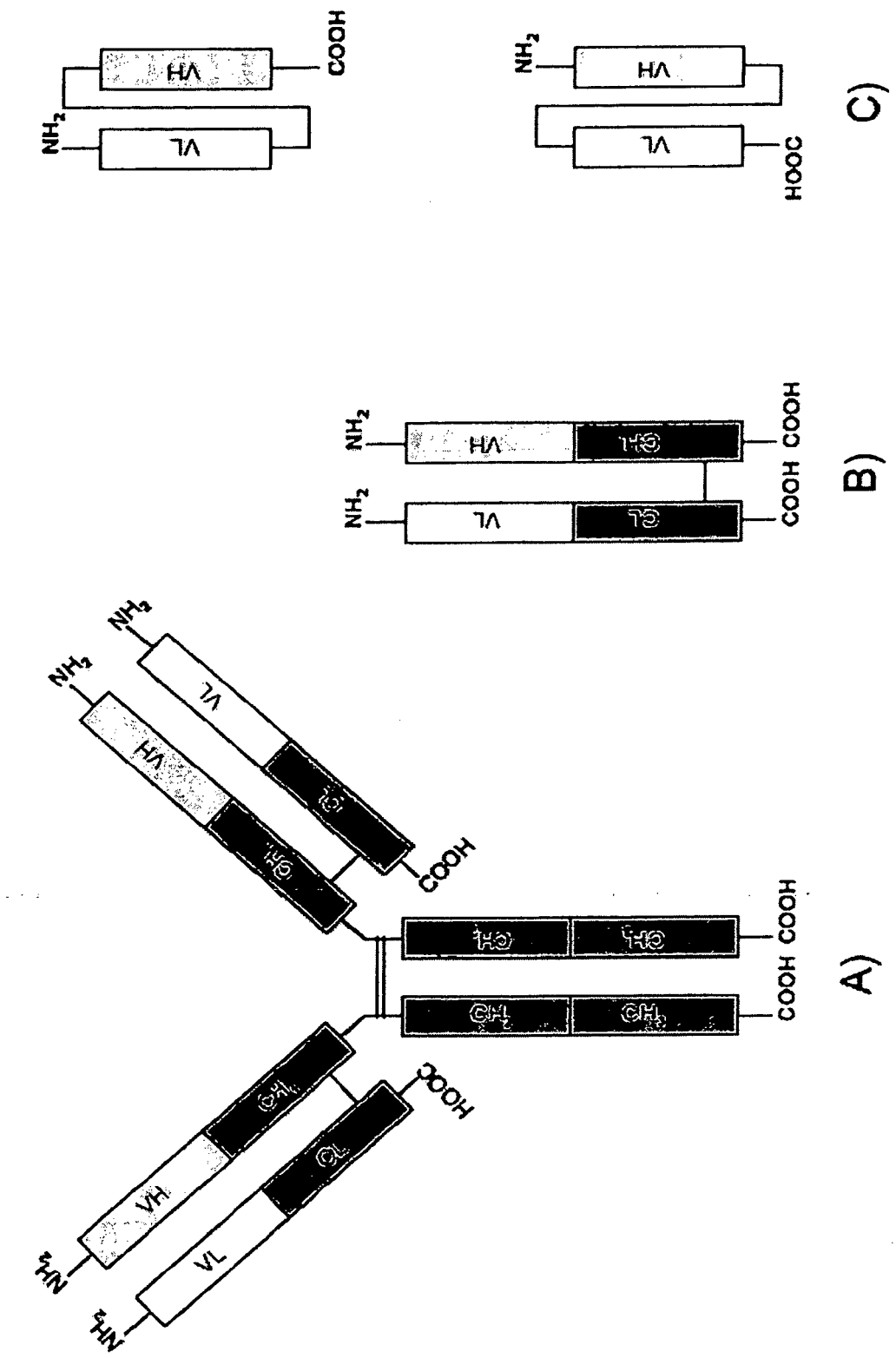

Fig. 5

T7 promotor
ATG
Signal peptid
VH
CH
Stop
SIRES
ATG
Signal peptid
VL
CL
Stop
muag
A70C30

Fig. 6

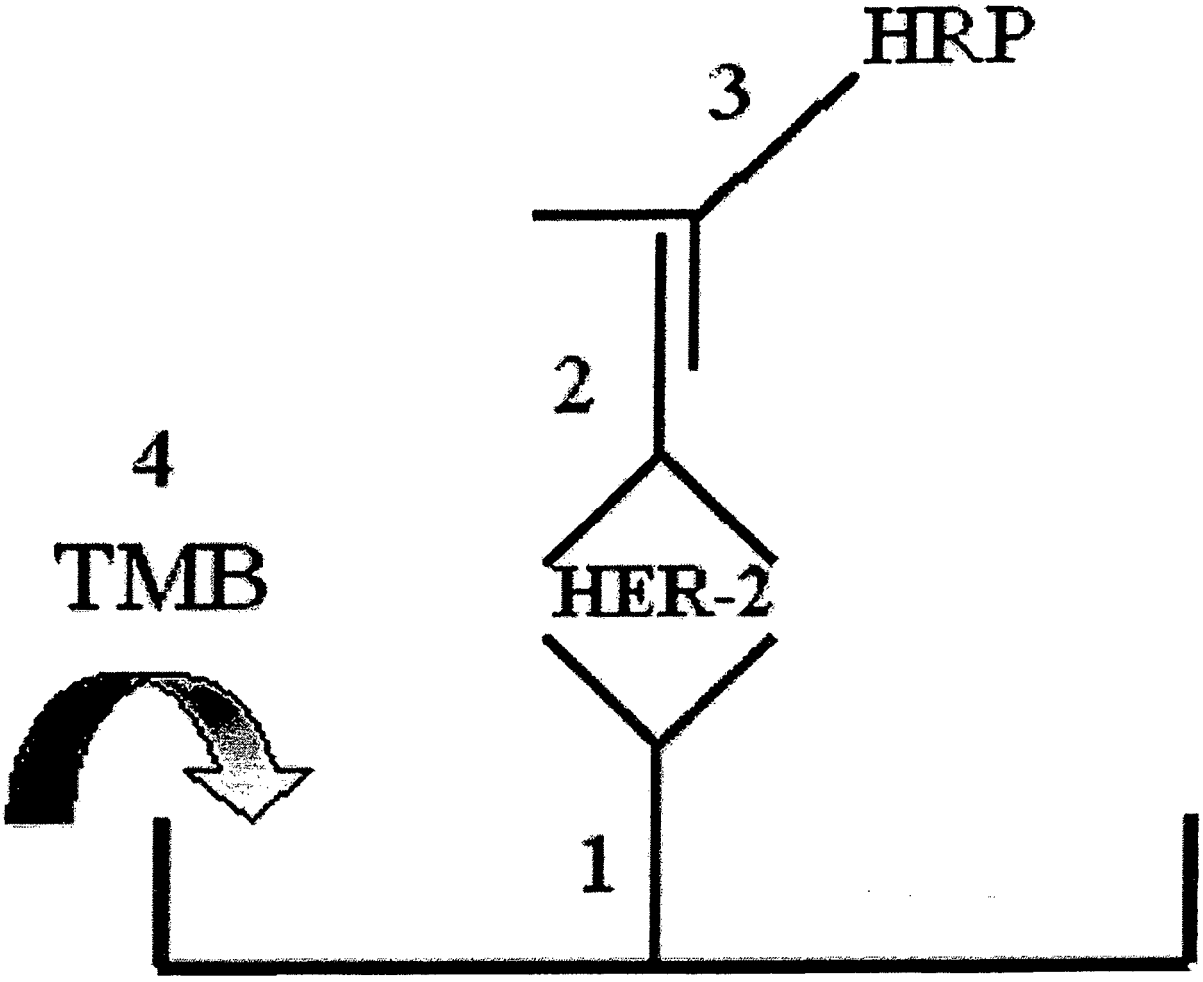

Fig. 7

CAG GCG TAT CTG CAG CAG AGC GGC GCG GAA CTG GTG CGC CCG
GGC GCG AGC GTG AAA ATG AGC TGC AAA GCG AGC GGC TAT ACC
TTT ACC AGC TAT AAC ATG CAT TGG GTG AAA CAG ACC CCG CGC
CAG GGC CTG GAA TGG ATT GGC GCG ATT TAT CCG GGC AAC GGC
GAT ACC AGC TAT AAC CAG AAA TTT AAA GGC AAA GCG ACC CTG
ACC GTG GAT AAA AGC AGC AGC ACC GCG TAT ATG CAG CTG AGC
AGC CTG ACC AGC GAA GAT AGC GCG GTG TAT TTT TGC GCG CGC
GTG GTG TAT TAT AGC AAC AGC TAT TGG TAT TTT GAT GTG TGG
GGC ACC GGC ACC ACC GTG ACC GTG AGC GGC CCG AGC GTG TTT
CCG CTG GCG CCG AGC AGC AAA AGC ACC AGC GGC GGC ACC GCG
GCG CTG GGC TGC CTG GTG AAA GAT TAT TTT CCG GAA CCG GTG
ACC GTG AGC TGG AAC AGC GGC GCG CTG ACC AGC GGC GTG CAT
ACC TTT CCG GCG GTG CTG CAG AGC AGC GGC CTG TAT AGC CTG
AGC AGC GTG GTG ACC GTG CCG AGC AGC AGC CTG GGC ACC CAG
ACC TAT ATT TGC AAC GTG AAC CAT AAA CCG AGC AAC ACC AAA
GTG GAT AAA AAA GCG GAA CCG AAA AGC TGC GAT AAA ACC CAT
ACC TGC CCG CCG TGC CCG GCG CCG GAA CTG CTG GGC GGC CCG
AGC GTG TTT CTG TTT CCG CCG AAA CCG AAA GAT ACC CTG ATG
ATT AGC CGC ACC CCG GAA GTG ACC TGC GTG GTG GTG GAT GTG
AGC CAT GAA GAT CCG GAA GTG AAA TTT AAC TGG TAT GTG GAT
GGC GTG GAA GTG CAT AAC GCG AAA ACC AAA CCG CGC GAA GAA
CAG TAT AAC AGC ACC TAT CGC GTG GTG AGC GTG CTG ACC GTG
CTG CAT CAG GAT TGG CTG AAC GGC AAA GAA TAT AAA TGC AAA
GTG AGC AAC AAA GCG CTG CCG GCG CCG ATT GAA AAA ACC ATT
AGC AAA GCG AAA GGC CAG CCG CGC GAA CCG CAG GTG TAT ACC
CTG CCG CCG AGC CGC GAT GAA CTG ACC AAA AAC CAG GTG AGC
CTG ACC TGC CTG GTG AAA GGC TTT TAT CCG AGC GAT ATT GCG
GTG GAA TGG GAA AGC AAC GGC CAG CCG GAA AAC AAC TAT AAA
ACC ACC CCG CCG GTG CTG GAT AGC GAT GGC AGC TTT TTT CTG
TAT AGC AAA CTG ACC GTG GAT AAA AGC CGC TGG CAG CAG GGC
AAC GTG TTT AGC TGC AGC GTG ATG CAT GAA GCG CTG CAT AAC
CAT TAT ACC CAG AAA AGC CTG AGC CTG AGC CCG GGC AAA TAA

Fig. 8

CAG GCC TAC CTG CAG CAG AGC GGC GCG GAG CTC GTG CGG CCG
GGG GCC TCG GTC AAG ATG AGC TGC AAG GCC AGC GGC TAC ACC
TTC ACG AGC TAC AAC ATG CAC TGG GTG AAG CAG ACC CCG CGC
CAG GGG CTG GAG TGG ATC GGC GCC ATC TAC CCC GGG AAC GGC
GAC ACC AGC TAC AAC CAG AAG TTC AAG GGC AAG GCG ACC CTG
ACG GTG GAC AAG TCG AGC AGC ACC GCC TAC ATG CAG CTC AGC
AGC CTG ACC TCG GAG GAC AGC GCC GTC TAC TTC TGC GCC CGG
GTG GTG TAC TAC AGC AAC AGC TAC TGG TAC TTC GAC GTC TGG
GGG ACC GGC ACG ACC GTG ACC GTG AGC GGG CCC AGC GTC TTC
CCC CTG GCC CCC TCG AGC AAG AGC ACC AGC GGC GGC ACG GCG
GCC CTC GGG TGC CTG GTG AAG GAC TAC TTC CCC GAG CCC GTG
ACC GTC AGC TGG AAC TCG GGC GCC CTG ACC AGC GGG GTG CAC
ACC TTC CCG GCC GTG CTC CAG AGC AGC GGC CTG TAC AGC CTG
AGC TCG GTC GTG ACG GTG CCC AGC AGC AGC CTC GGG ACC CAG
ACC TAC ATC TGC AAC GTC AAC CAC AAG CCC AGC AAC ACC AAG
GTG GAC AAG AAG GCG GAG CCC AAG TCG TGC GAC AAG ACG CAC
ACC TGC CCG CCC TGC CCC GCC CCC GAG CTG CTG GGC GGC CCG
AGC GTG TTC CTC TTC CCG CCC AAG CCC AAG GAC ACC CTG ATG
ATC AGC CGC ACC CCC GAG GTC ACG TGC GTG GTG GTC GAC GTG
AGC CAC GAG GAC CCC GAG GTG AAG TTC AAC TGG TAC GTC GAC
GGG GTG GAG GTG CAC AAC GCC AAG ACC AAG CCC CGG GAG GAG
CAG TAC AAC AGC ACC TAC CGC GTC GTG AGC GTG CTG ACC GTC
CTC CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG
GTG TCG AAC AAG GCC CTG CCG GCC CCC ATC GAG AAG ACG ATC
AGC AAG GCG AAG GGG CAG CCC CGG GAG CCC CAG GTG TAC ACC
CTC CCG CCC AGC CGC GAC GAG CTG ACC AAG AAC CAG GTC AGC
CTG ACC TGC CTC GTG AAG GGC TTC TAC CCC AGC GAC ATC GCC
GTG GAG TGG GAG TCG AAC GGG CAG CCC GAG AAC AAC TAC AAG
ACG ACC CCG CCC GTC CTG GAC AGC GAC GGC AGC TTC TTC CTG
TAC AGC AAG CTC ACC GTG GAC AAG AGC CGG TGG CAG CAG GGC
AAC GTG TTC AGC TGC TCG GTC ATG CAC GAG GCC CTG CAC AAC
CAC TAC ACC CAG AAG AGC CTG AGC CTC AGC CCC GGG AAG TGA

Fig. 9

CAG ATT GTG CTG AGC CAG AGC CCG GCG ATT CTG AGC GCG AGC
CCG GGC GAA AAA GTG ACC ATG ACC TGC CGC GCG AGC AGC AGC
GTG AGC TAT ATG CAT TGG TAT CAG CAG AAA CCG GGC AGC AGC
CCG AAA CCG TGG ATT TAT GCG CCG AGC AAC CTG GCG AGC GGC
GTG CCG GCG CGC TTT AGC GGC AGC GGC AGC GGC ACC AGC TAT
AGC CTG ACC ATT AGC CGC GTG GAA GCG GAA GAT GCG GCG ACC
TAT TAT TGC CAG CAG TGG AGC TTT AAC CCG CCG ACC TTT GGC
GCG GGC ACC AAA CTG GAA CTG AAA CGC ACC GTG GCG GCG CCG
AGC GTG TTT ATT TTT CCG CCG AGC GAT GAA CAG CTG AAA AGC
GGC ACC GCG AGC GTG GTG TGC CTG CTG AAC AAC TTT TAT CCG
CGC GAA GCG AAA GTG CAG TGG AAA GTG GAT AAC GCG CTG CAG
AGC GGC AAC AGC CAG GAA AGC GTG ACC GAA CAG GAT AGC AAA
GAT AGC ACC TAT AGC CTG AGC AGC ACC CTG ACC CTG AGC AAA
GCG GAT TAT GAA AAA CAT AAA GTG TAT GCG TGC GAA GTG ACC
CAT CAG GGC CTG AGC AGC CCG GTG ACC AAA AGC TTT AAC CGC
TAA

Fig. 10

CAG ATC GTG CTG AGC CAG TCG CCG GCC ATC CTC AGC GCG AGC
CCC GGC GAG AAG GTC ACC ATG ACG TGC CGG GCC AGC AGC TCG
GTG AGC TAC ATG CAC TGG TAC CAG CAG AAG CCC GGG AGC AGC
CCC AAG CCG TGG ATC TAC GCC CCC AGC AAC CTG GCC TCG GGC
GTG CCC GCG CGC TTC AGC GGG AGC GGC AGC GGG ACC AGC TAC
AGC CTG ACC ATC TCG CGG GTC GAG GCC GAG GAC GCC GCC ACC
TAC TAC TGC CAG CAG TGG AGC TTC AAC CCG CCC ACG TTC GGC
GCC GGC ACC AAG CTC GAG CTG AAG CGC ACC GTG GCG GCC CCC
AGC GTG TTC ATC TTC CCG CCC AGC GAC GAG CAG CTG AAG AGC
GGG ACC GCC TCG GTC GTG TGC CTC CTG AAC AAC TTC TAC CCC
CGG GAG GCC AAG GTG CAG TGG AAG GTC GAC AAC GCG CTG CAG
AGC GGC AAC AGC CAG GAG AGC GTG ACG GAG CAG GAC AGC AAG
GAC AGC ACC TAC TCG CTC AGC AGC ACC CTG ACC CTG AGC AAG
GCC GAC TAC GAG AAG CAC AAG GTG TAC GCC TGC GAG GTC ACG
CAC CAG GGG CTC AGC TCG CCC GTG ACC AAG AGC TTC AAC CGC
TGA

Fig. 11

AAGCTTACC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCC
TCGCCCTGACGCAGACCTGGGCCGGGCAGGCCTACCTGCAGCAGAGCGGCGCGGAGCT
CGTGCGGCCGGGGGCCTCGGTCAAGATGAGCTGCAAGGCCAGCGGCTACACCTTCACG
AGCTACAACATGCACTGGGTGAAGCAGACCCCGCGCCAGGGGCTGGAGTGGATCGGCG
CCATCTACCCCGGGAACGGCGACACCAGCTACAACCAGAAGTTCAAGGGCAAGGCGAC
CCTGACGGTGGACAAGTCGAGCAGCACCGCCTACATGCAGCTCAGCAGCCTGACCTCG
GAGGACAGCGCCGTCTACTTCTGCGCCCGGGTGGTGTACTACAGCAACAGCTACTGGT
ACTTCGACGTCTGGGGGACCGGCACGACCGTGACCGTGAGCGGGCCCAGCGTCTTCCC
CCTGGCCCCCTCGAGCAAGAGCACCAGCGGCGGCACGGCGGCCCTCGGGTGCCTGGTG
AAGGACTACTTCCCCGAGCCCGTGACCGTCAGCTGGAACTCGGGCGCCCTGACCAGCG
GGGTGCACACCTTCCCGGCCGTGCTCCAGAGCAGCGGCCTGTACAGCCTGAGCTCGGT
CGTGACGGTGCCCAGCAGCAGCCTCGGGACCCAGACCTACATCTGCAACGTCAACCAC
AAGCCCAGCAACACCAAGGTGGACAAGAAGGCGGAGCCCAAGTCGTGCGACAAGACGC
ACACCTGCCCGCCCTGCCCCGCCCCCGAGCTGCTGGGCGGCCCGAGCGTGTTCCTCTT
CCCGCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCCGAGGTCACGTGCGTG
GTGGTCGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTCGACGGGG
TGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTACAACAGCACCTACCG
CGTCGTGAGCGTGCTGACCGTCCTCCACCAGGACTGGCTGAACGGCAAGGAGTACAAG
TGCAAGGTGTCGAACAAGGCCCTGCCGGCCCCCATCGAGAAGACGATCAGCAAGGCGA
AGGGGCAGCCCCGGGAGCCCCAGGTGTACACCCTCCCGCCCAGCCGCGACGAGCTGAC
CAAGAACCAGGTCAGCCTGACCTGCCTCGTGAAGGGCTTCTACCCCAGCGACATCGCC
GTGGAGTGGGAGTCGAACGGGCAGCCCGAGAACAACTACAAGACGACCCCGCCCGTCC
TGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTCACCGTGGACAAGAGCCGGTG
GCAGCAGGGCAACGTGTTCAGCTGCTCGGTCATGCACGAGGCCCTGCACAACCACTAC
ACCCAGAAGAGCCTGAGCCTCAGCCCCGGGAAGCATCATCATCATCATCATTGACC**AG**
ATCTTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACAT
TTCTGACATTTCTGACATTTCTGACATTTCTGACATATGCATACC***ATG***GCCGTGATGG
CGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGACGCAGACCTGGGC
CGGGCAGATCGTGCTGAGCCAGTCGCCGGCCATCCTCAGCGCGAGCCCCGGCGAGAAG
GTCACCATGACGTGCCGGGCCAGCAGCTCGGTGAGCTACATGCACTGGTACCAGCAGA
AGCCCGGGAGCAGCCCCAAGCCGTGGATCTACGCCCCCAGCAACCTGGCCTCGGGCGT
GCCCGCGCGCTTCAGCGGGAGCGGCAGCGGGACCAGCTACAGCCTGACCATCTCGCGG
GTCGAGGCCGAGGACGCCGCCACCTACTACTGCCAGCAGTGGAGCTTCAACCCGCCCA
CGTTCGGCGCCGGCACCAAGCTCGAGCTGAAGCGCACCGTGGCGGCCCCCAGCGTGTT
CATCTTCCCGCCCAGCGACGAGCAGCTGAAGAGCGGGACCGCCTCGGTCGTGTGCCTC
CTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTCGACAACGCGCTGC
AGAGCGGCAACAGCCAGGAGAGCGTGACGGAGCAGGACAGCAAGGACAGCACCTACTC
GCTCAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCC
TGCGAGGTCACGCACCAGGGGCTCAGCTCGCCCGTGACCAAGAGCTTCAACCGCTGAC
CACTAGT

Fig. 12

CAG GTG CAG CTG AAA CAG AGC GGC CCG GGC CTG GTG CAG CCG
AGC CAG AGC CTG AGC ATT ACC TGC ACC GTG AGC GGC TTT AGC
CTG ACC AAC TAT GGC GTG CAT TGG GTG CGC CAG AGC CCG GGC
AAA GGC CTG GAA TGG CTG GGC GTG ATT TGG AGC GGC GGC AAC
ACC GAT TAT AAC ACC CCG TTT ACC AGC CGC CTG AGC ATT AAC
AAA GAT AAC AGC AAA AGC CAG GTG TTT TTT AAA ATG AAC AGC
CTG CAG AGC AAC GAT ACC GCG ATT TAT TAT TGC GCG CGC GCG
CTG ACC TAT TAT GAT TAT GAA TTT GCG TAT TGG GGC CAG GGC
ACC CTG GTG ACC GTG AGC GCG GCG AGC ACC AAA GGC CCG AGC
GTG TTT CCG CTG GCG CCG AGC AGC AAA AGC ACC AGC GGC GGC
ACC GCG GCG CTG GGC TGC CTG GTG AAA GAT TAT TTT CCG GAA
CCG GTG ACC GTG AGC TGG AAC AGC GGC GCG CTG ACC AGC GGC
GTG CAT ACC TTT CCG GCG GTG CTG CAG AGC AGC GGC CTG TAT
AGC CTG AGC AGC GTG GTG ACC GTG CCG AGC AGC AGC CTG GGC
ACC CAG ACC TAT ATT TGC AAC GTG AAC CAT AAA CCG AGC AAC
ACC AAA GTG GAT AAA CGC GTG GAA CCG AAA AGC CCG AAA AGC
TGC GAT AAA ACC CAT ACC TGC CCG CCG TGC CCG GCG CCG GAA
CTG CTG GGC GGC CCG AGC GTG TTT CTG TTT CCG CCG AAA CCG
AAA GAT ACC CTG ATG ATT AGC CGC ACC CCG GAA GTG ACC TGC
GTG GTG GTG GAT GTG AGC CAT GAA GAT CCG GAA GTG AAA TTT
AAC TGG TAT GTG GAT GGC GTG GAA GTG CAT AAC GCG AAA ACC
AAA CCG CGC GAA GAA CAG TAT AAC AGC ACC TAT CGC GTG GTG
AGC GTG CTG ACC GTG CTG CAT CAG GAT TGG CTG AAC GGC AAA
GAA TAT AAA TGC AAA GTG AGC AAC AAA GCG CTG CCG GCG CCG
ATT GAA AAA ACC ATT AGC AAA GCG AAA GGC CAG CCG CGC GAA
CCG CAG GTG TAT ACC CTG CCG CCG AGC CGC GAT GAA CTG ACC
AAA AAC CAG GTG AGC CTG ACC TGC CTG GTG AAA GGC TTT TAT
CCG AGC GAT ATT GCG GTG GAA TGG GAA AGC AAC GGC CAG CCG
GAA AAC AAC TAT AAA ACC ACC CCG CCG GTG CTG GAT AGC GAT
GGC AGC TTT TTT CTG TAT AGC AAA CTG ACC GTG GAT AAA AGC
CGC TGG CAG CAG GGC AAC GTG TTT AGC TGC AGC GTG ATG CAT
GAA GCG CTG CAT AAC CAT TAT ACC CAG AAA AGC CTG AGC CTG
AGC CCG GGC AAA TAA

Fig. 13

CAG GTG CAG CTG AAG CAG AGC GGC CCG GGG CTC GTC CAG CCC
TCG CAG AGC CTG AGC ATC ACC TGC ACG GTG AGC GGC TTC AGC
CTG ACC AAC TAC GGG GTG CAC TGG GTC CGG CAG TCG CCC GGC
AAG GGG CTC GAG TGG CTG GGC GTG ATC TGG AGC GGC GGG AAC
ACC GAC TAC AAC ACC CCC TTC ACG AGC CGC CTG AGC ATC AAC
AAG GAC AAC AGC AAG TCG CAG GTG TTC TTC AAG ATG AAC AGC
CTC CAG AGC AAC GAC ACC GCC ATC TAC TAC TGC GCG CGG GCC
CTG ACC TAC TAC GAC TAC GAG TTC GCC TAC TGG GGC CAG GGG
ACC CTG GTC ACG GTG AGC GCC GCG AGC ACC AAG GGC CCG AGC
GTG TTC CCC CTC GCC CCC TCG AGC AAG AGC ACC AGC GGC GGG
ACC GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAG
CCG GTG ACG GTG AGC TGG AAC TCG GGG GCC CTC ACC AGC GGC
GTC CAC ACC TTC CCC GCG GTG CTG CAG AGC AGC GGG CTG TAC
AGC CTC AGC TCG GTG GTC ACC GTG CCC AGC AGC AGC CTG GGC
ACG CAG ACC TAC ATC TGC AAC GTG AAC CAC AAG CCC AGC AAC
ACC AAG GTC GAC AAG CGC GTG GAG CCG AAG TCG CCC AAG AGC
TGC GAC AAG ACC CAC ACG TGC CCG CCC TGC CCC GCC CCC GAG
CTG CTC GGC GGG CCC AGC GTG TTC CTG TTC CCG CCC AAG CCC
AAG GAC ACC CTG ATG ATC AGC CGG ACC CCC GAG GTC ACC TGC
GTG GTG GTC GAC GTG AGC CAC GAG GAC CCG GAG GTG AAG TTC
AAC TGG TAC GTC GAC GGC GTG GAG GTG CAC AAC GCC AAG ACG
AAG CCC CGC GAG GAG CAG TAC AAC AGC ACC TAC CGG GTC GTG
TCG GTG CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGG AAG
GAG TAC AAG TGC AAG GTG AGC AAC AAG GCC CTC CCC GCG CCC
ATC GAG AAG ACC ATC AGC AAG GCC AAG GGC CAG CCG CGC GAG
CCC CAG GTG TAC ACG CTG CCC CCC AGC CGG GAC GAG CTG ACC
AAG AAC CAG GTC AGC CTC ACC TGC CTG GTG AAG GGG TTC TAC
CCG TCG GAC ATC GCC GTG GAG TGG GAG AGC AAC GGC CAG CCC
GAG AAC AAC TAC AAG ACC ACG CCC CCG GTC CTG GAC AGC GAC
GGC AGC TTC TTC CTC TAC AGC AAG CTG ACC GTG GAC AAG AGC
CGC TGG CAG CAG GGG AAC GTG TTC TCG TGC AGC GTC ATG CAC
GAG GCC CTG CAC AAC CAC TAC ACC CAG AAG AGC CTC AGC CTG
AGC CCC GGC AAG TGA

Fig. 14

```
GAT ATT CTG CTG ACC CAG AGC CCG GTG ATT CTG AGC GTG AGC
CCG GGC GAA CGC GTG AGC TTT AGC TGC CGC GCG AGC CAG AGC
ATT GGC ACC AAC ATT CAT TGG TAT CAG CAG CGC ACC AAC GGC
AGC CCG CGC CTG CTG ATT AAA TAT GCG AGC GAA AGC ATT AGC
GGC ATT CCG AGC CGC TTT AGC GGC AGC GGC AGC GGC ACC GAT
TTT ACC CTG AGC ATT AAC AGC GTG GAA AGC GAA GAT ATT GCG
GAT TAT TAT TGC CAG CAG AAC AAC AAC TGG CCG ACC ACC TTT
GGC GCG GGC ACC AAA CTG GAA CTG AAA CGC ACC GTG GCG GCG
CCG AGC GTG TTT ATT TTT CCG CCG AGC GAT GAA CAG CTG AAA
AGC GGC ACC GCG AGC GTG GTG TGC CTG CTG AAC AAC TTT TAT
CCG CGC GAA GCG AAA GTG CAG TGG AAA GTG GAT AAC GCG CTG
CAG AGC GGC AAC AGC CAG GAA AGC GTG ACC GAA CAG GAT AGC
AAA GAT AGC ACC TAT AGC CTG AGC AGC ACC CTG ACC CTG AGC
AAA GCG GAT TAT GAA AAA CAT AAA GTG TAT GCG TGC GAA GTG
ACC CAT CAG GGC CTG AGC AGC CCG GTG ACC AAA AGC TTT AAC
CGC GGC GCG TAA
```

Fig. 15

GAC ATC CTG CTC ACC CAG AGC CCG GTG ATC CTG TCG GTC AGC
CCC GGC GAG CGG GTG AGC TTC AGC TGC CGC GCC AGC CAG TCG
ATC GGG ACG AAC ATC CAC TGG TAC CAG CAG CGG ACC AAC GGC
AGC CCC CGC CTG CTC ATC AAG TAC GCG AGC GAG AGC ATC AGC
GGG ATC CCC TCG CGG TTC AGC GGC AGC GGG AGC GGC ACC GAC
TTC ACC CTG AGC ATC AAC AGC GTG GAG TCG GAG GAC ATC GCC
GAC TAC TAC TGC CAG CAG AAC AAC AAC TGG CCG ACG ACC TTC
GGC GCC GGG ACC AAG CTG GAG CTC AAG CGC ACC GTC GCC GCG
CCC AGC GTG TTC ATC TTC CCG CCC AGC GAC GAG CAG CTG AAG
AGC GGC ACG GCC AGC GTG GTC TGC CTG CTC AAC AAC TTC TAC
CCC CGG GAG GCC AAG GTG CAG TGG AAG GTG GAC AAC GCC CTG
CAG TCG GGG AAC AGC CAG GAG AGC GTC ACC GAG CAG GAC AGC
AAG GAC AGC ACC TAC AGC CTG TCG AGC ACC CTC ACG CTG AGC
AAG GCC GAC TAC GAG AAG CAC AAG GTG TAC GCG TGC GAG GTG
ACC CAC CAG GGC CTG AGC AGC CCC GTC ACC AAG TCG TTC AAC
CGC GGC GCC TGA

Fig. 16

AAGCTTACC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCC
TCGCCCTGACGCAGACCTGGGCCGGGCAGGTGCAGCTGAAGCAGAGCGGCCCGGGGCT
CGTCCAGCCCTCGCAGAGCCTGAGCATCACCTGCACGGTGAGCGGCTTCAGCCTGACC
AACTACGGGGTGCACTGGGTCCGGCAGTCGCCCGGCAAGGGGCTCGAGTGGCTGGGCG
TGATCTGGAGCGGCGGGAACACCGACTACAACACCCCCTTCACGAGCCGCCTGAGCAT
CAACAAGGACAACAGCAAGTCGCAGGTGTTCTTCAAGATGAACAGCCTCCAGAGCAAC
GACACCGCCATCTACTACTGCGCGCGGGCCCTGACCTACTACGACTACGAGTTCGCCT
ACTGGGGCCAGGGGACCCTGGTCACGGTGAGCGCCGCGAGCACCAAGGGCCCGAGCGT
GTTCCCCCTCGCCCCCTCGAGCAAGAGCACCAGCGGCGGGACCGCCGCCCTGGGCTGC
CTGGTCAAGGACTACTTCCCCGAGCCGGTGACGGTGAGCTGGAACTCGGGGGCCCTCA
CCAGCGGCGTCCACACCTTCCCCGCGGTGCTGCAGAGCAGCGGGCTGTACAGCCTCAG
CTCGGTGGTCACCGTGCCCAGCAGCAGCCTGGGCACGCAGACCTACATCTGCAACGTG
AACCACAAGCCCAGCAACACCAAGGTCGACAAGCGCGTGGAGCCGAAGTCGCCCAAGA
GCTGCGACAAGACCCACACGTGCCCGCCCTGCCCCGCCCCCGAGCTGCTCGGCGGGCC
CAGCGTGTTCCTGTTCCCGCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCC
GAGGTCACCTGCGTGGTGGTCGACGTGAGCCACGAGGACCCGGAGGTGAAGTTCAACT
GGTACGTCGACGGCGTGGAGGTGCACAACGCCAAGACGAAGCCCCGCGAGGAGCAGTA
CAACAGCACCTACCGGGTCGTGTCGGTGCTCACCGTCCTGCACCAGGACTGGCTGAAC
GGGAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTCCCCGCGCCCATCGAGAAGA
CCATCAGCAAGGCCAAGGGCCAGCCGCGCGAGCCCCAGGTGTACACGCTGCCCCCCAG
CCGGGACGAGCTGACCAAGAACCAGGTCAGCCTCACCTGCCTGGTGAAGGGGTTCTAC
CCGTCGGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGA
CCACGCCCCCGGTCCTGGACAGCGACGGCAGCTTCTTCCTCTACAGCAAGCTGACCGT
GGACAAGAGCCGCTGGCAGCAGGGGAACGTGTTCTCGTGCAGCGTCATGCACGAGGCC
CTGCACAACCACTACACCCAGAAGAGCCTCAGCCTGAGCCCCGGCAAGCATCATCATC
ATCATCATTGACC**AGATCTTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTC**
TGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATATGCATA
CC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCT
GACGCAGACCTGGGCCGGGGACATCCTGCTCACCCAGAGCCCGGTGATCCTGTCGGTC
AGCCCCGGCGAGCGGGTGAGCTTCAGCTGCCGCGCCAGCCAGTCGATCGGGACGAACA
TCCACTGGTACCAGCAGCGGACCAACGGCAGCCCCCGCCTGCTCATCAAGTACGCGAG
CGAGAGCATCAGCGGGATCCCCTCGCGGTTCAGCGGCAGCGGGAGCGGCACCGACTTC
ACCCTGAGCATCAACAGCGTGGAGTCGGAGGACATCGCCGACTACTACTGCCAGCAGA
ACAACAACTGGCCGACGACCTTCGGCGCCGGGACCAAGCTGGAGCTCAAGCGCACCGT
CGCCGCGCCCAGCGTGTTCATCTTCCCGCCCAGCGACGAGCAGCTGAAGAGCGGCACG
GCCAGCGTGGTCTGCCTGCTCAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGA
AGGTGGACAACGCCCTGCAGTCGGGGAACAGCCAGGAGAGCGTCACCGAGCAGGACAG
CAAGGACAGCACCTACAGCCTGTCGAGCACCCTCACGCTGAGCAAGGCCGACTACGAG
AAGCACAAGGTGTACGCGTGCGAGGTGACCCACCAGGGCCTGAGCAGCCCCGTCACCA
AGTCGTTCAACCGCGGCGCCTGACCACTAGT

Fig. 17

GAA GTG CAG CTG GTG GAA AGC GGC GGC GGC CTG GTG CAG CCG
GGC GGC AGC CTG CGC CTG AGC TGC GCG GCG AGC GGC TTT AAC
ATT AAA GAT ACC TAT ATT CAT TGG GTG CGC CAG GCG CCG GGC
AAA GGC CTG GAA TGG GTG GCG CGC ATT TAT CCG ACC AAC GGC
TAT ACC CGC TAT GCG GAT AGC GTG AAA GGC CGC TTT ACC ATT
AGC GCG GAT ACC AGC AAA AAC ACC GCG TAT CTG CAG ATG AAC
AGC CTG CGC GCG GAA GAT ACC GCG GTG TAT TAT TGC AGC CGC
TGG GGC GGC GAT GGC TTT TAT GCG ATG GAT TAT TGG GGC CAG
GGC ACC CTG GTG ACC GTG AGC AGC GCG AGC ACC AAA GGC CCG
AGC GTG TTT CCG CTG GCG CCG AGC AGC AAA AGC ACC AGC GGC
GGC ACC GCG GCG CTG GGC TGC CTG GTG AAA GAT TAT TTT CCG
GAA CCG GTG ACC GTG AGC TGG AAC AGC GGC GCG CTG ACC AGC
GGC GTG CAT ACC TTT CCG GCG GTG CTG CAG AGC AGC GGC CTG
TAT AGC CTG AGC AGC GTG GTG ACC GTG CCG AGC AGC AGC CTG
GGC ACC CAG ACC TAT ATT TGC AAC GTG AAC CAT AAA CCG AGC
AAC ACC AAA GTG GAT AAA AAA GTG GAA CCG CCG AAA AGC TGC
GAT AAA ACC CAT ACC TGC CCG CCG TGC CCG GCG CCG GAA CTG
CTG GGC GGC CCG AGC GTG TTT CTG TTT CCG CCG AAA CCG AAA
GAT ACC CTG ATG ATT AGC CGC ACC CCG GAA GTG ACC TGC GTG
GTG GTG GAT GTG AGC CAT GAA GAT CCG GAA GTG AAA TTT AAC
TGG TAT GTG GAT GGC GTG GAA GTG CAT AAC GCG AAA ACC AAA
CCG CGC GAA GAA CAG TAT AAC AGC ACC TAT CGC GTG GTG AGC
GTG CTG ACC GTG CTG CAT CAG GAT TGG CTG AAC GGC AAA GAA
TAT AAA TGC AAA GTG AGC AAC AAA GCG CTG CCG GCG CCG ATT
GAA AAA ACC ATT AGC AAA GCG AAA GGC CAG CCG CGC GAA CCG
CAG GTG TAT ACC CTG CCG CCG AGC CGC GAT GAA CTG ACC AAA
AAC CAG GTG AGC CTG ACC TGC CTG GTG AAA GGC TTT TAT CCG
AGC GAT ATT GCG GTG GAA TGG GAA AGC AAC GGC CAG CCG GAA
AAC AAC TAT AAA ACC ACC CCG CCG GTG CTG GAT AGC GAT GGC
AGC TTT TTT CTG TAT AGC AAA CTG ACC GTG GAT AAA AGC CGC
TGG CAG CAG GGC AAC GTG TTT AGC TGC AGC GTG ATG CAT GAA
GCG CTG CAT AAC CAT TAT ACC CAG AAA AGC CTG AGC CTG AGC
CCG GGC AAA TAA

Fig. 18

GAG GTG CAG CTG GTC GAG AGC GGC GGG GGC CTC GTG CAG CCG
GGC GGG TCG CTG CGG CTG AGC TGC GCC GCG AGC GGG TTC AAC
ATC AAG GAC ACC TAC ATC CAC TGG GTG CGC CAG GCC CCC GGC
AAG GGC CTC GAG TGG GTC GCC CGG ATC TAC CCC ACG AAC GGG
TAC ACC CGC TAC GCC GAC AGC GTG AAG GGC CGG TTC ACC ATC
AGC GCG GAC ACC TCG AAG AAC ACG GCC TAC CTG CAG ATG AAC
AGC CTG CGC GCC GAG GAC ACC GCC GTG TAC TAC TGC AGC CGG
TGG GGC GGC GAC GGG TTC TAC GCC ATG GAC TAC TGG GGG CAG
GGC ACC CTC GTC ACC GTG AGC AGC GCG TCG ACG AAG GGG CCC
AGC GTG TTC CCG CTG GCC CCC AGC AGC AAG AGC ACC AGC GGC
GGG ACC GCC GCC CTG GGC TGC CTC GTC AAG GAC TAC TTC CCC
GAG CCC GTG ACC GTG TCG TGG AAC AGC GGC GCG CTG ACG AGC
GGG GTC CAC ACC TTC CCG GCC GTG CTG CAG AGC AGC GGC CTC
TAC TCG CTG AGC AGC GTG GTC ACC GTG CCC AGC AGC AGC CTG
GGG ACC CAG ACG TAC ATC TGC AAC GTG AAC CAC AAG CCC TCG
AAC ACC AAG GTC GAC AAG AAG GTG GAG CCC CCG AAG AGC TGC
GAC AAG ACC CAC ACC TGC CCG CCC TGC CCC GCC CCC GAG CTC
CTG GGC GGG CCC AGC GTG TTC CTG TTC CCG CCC AAG CCC AAG
GAC ACG CTC ATG ATC AGC CGC ACC CCC GAG GTC ACC TGC GTG
GTG GTC GAC GTG AGC CAC GAG GAC CCC GAG GTG AAG TTC AAC
TGG TAC GTC GAC GGC GTG GAG GTG CAC AAC GCC AAG ACC AAG
CCG CGG GAG GAG CAG TAC AAC TCG ACG TAC CGC GTC GTG AGC
GTG CTG ACC GTC CTG CAC CAG GAC TGG CTC AAC GGC AAG GAG
TAC AAG TGC AAG GTG AGC AAC AAG GCC CTG CCC GCG CCC ATC
GAG AAG ACC ATC AGC AAG GCC AAG GGG CAG CCC CGG GAG CCG
CAG GTG TAC ACC CTG CCC CCC AGC CGC GAC GAG CTC ACG AAG
AAC CAG GTC AGC CTG ACC TGC CTG GTG AAG GGC TTC TAC CCC
TCG GAC ATC GCC GTG GAG TGG GAG AGC AAC GGG CAG CCG GAG
AAC AAC TAC AAG ACC ACC CCG CCC GTC CTC GAC AGC GAC GGC
AGC TTC TTC CTG TAC AGC AAG CTG ACG GTG GAC AAG TCG CGG
TGG CAG CAG GGC AAC GTG TTC AGC TGC AGC GTC ATG CAC GAG
GCC CTC CAC AAC CAC TAC ACC CAG AAG AGC CTG AGC CTG AGC
CCC GGG AAG TGA

Fig. 19

GAT ATT CAG ATG ACC CAG AGC CCG AGC AGC CTG AGC GCG AGC
GTG GGC GAT CGC GTG ACC ATT ACC TGC CGC GCG AGC CAG GAT
GTG AAC ACC GCG GTG GCG TGG TAT CAG CAG AAA CCG GGC AAA
GCG CCG AAA CTG CTG ATT TAT AGC GCG AGC TTT CTG TAT AGC
GGC GTG CCG AGC CGC TTT AGC GGC AGC CGC AGC GGC ACC GAT
TTT ACC CTG ACC ATT AGC AGC CTG CAG CCG GAA GAT TTT GCG
ACC TAT TAT TGC CAG CAG CAT TAT ACC ACC CCG CCG ACC TTT
GGC CAG GGC ACC AAA GTG GAA ATT AAA CGC ACC GTG GCG GCG
CCG AGC GTG TTT ATT TTT CCG CCG AGC GAT GAA CAG CTG AAA
AGC GGC ACC GCG AGC GTG GTG TGC CTG CTG AAC AAC TTT TAT
CCG CGC GAA GCG AAA GTG CAG TGG AAA GTG GAT AAC GCG CTG
CAG AGC GGC AAC AGC CAG GAA AGC GTG ACC GAA CAG GAT AGC
AAA GAT AGC ACC TAT AGC CTG AGC AGC ACC CTG ACC CTG AGC
AAA GCG GAT TAT GAA AAA CAT AAA GTG TAT GCG TGC GAA GTG
ACC CAT CAG GGC CTG AGC AGC CCG GTG ACC AAA AGC TTT AAC
CGC GGC GAA TGC TAA

Fig. 20

GAC ATC CAG ATG ACC CAG AGC CCG TCG AGC CTG AGC GCC AGC
GTG GGC GAC CGG GTC ACG ATC ACC TGC CGC GCG AGC CAG GAC
GTG AAC ACC GCC GTG GCC TGG TAC CAG CAG AAG CCC GGG AAG
GCC CCC AAG CTC CTG ATC TAC TCG GCG AGC TTC CTG TAC AGC
GGC GTC CCC AGC CGG TTC AGC GGG TCG CGC AGC GGC ACC GAC
TTC ACG CTC ACC ATC AGC AGC CTG CAG CCG GAG GAC TTC GCC
ACC TAC TAC TGC CAG CAG CAC TAC ACC ACG CCC CCC ACC TTC
GGG CAG GGC ACC AAG GTG GAG ATC AAG CGG ACC GTG GCC GCC
CCC AGC GTC TTC ATC TTC CCG CCC AGC GAC GAG CAG CTG AAG
TCG GGC ACG GCC AGC GTG GTG TGC CTC CTG AAC AAC TTC TAC
CCC CGC GAG GCG AAG GTC CAG TGG AAG GTG GAC AAC GCC CTG
CAG AGC GGG AAC AGC CAG GAG AGC GTG ACC GAG CAG GAC TCG
AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
AAG GCC GAC TAC GAG AAG CAC AAG GTC TAC GCC TGC GAG GTG
ACC CAC CAG GGG CTC TCG AGC CCC GTG ACC AAG AGC TTC AAC
CGG GGC GAG TGC TGA

Fig. 21

AAGCTTACC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCC
TCGCCCTGACGCAGACCTGGGCCGGGGAGGTGCAGCTGGTCGAGAGCGGCGGGGGCCT
CGTGCAGCCGGGCGGGTCGCTGCGGCTGAGCTGCGCCGCGAGCGGGTTCAACATCAAG
GACACCTACATCCACTGGGTGCGCCAGGCCCCCGGCAAGGGCCTCGAGTGGGTCGCCC
GGATCTACCCCACGAACGGGTACACCCGCTACGCCGACAGCGTGAAGGGCCGGTTCAC
CATCAGCGCGGACACCTCGAAGAACACGGCCTACCTGCAGATGAACAGCCTGCGCGCC
GAGGACACCGCCGTGTACTACTGCAGCCGGTGGGGCGGCGACGGGTTCTACGCCATGG
ACTACTGGGGGCAGGGCACCCTCGTCACCGTGAGCAGCGCGTCGACGAAGGGGCCCAG
CGTGTTCCCGCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGGACCGCCGCCCTGGGC
TGCCTCGTCAAGGACTACTTCCCCGAGCCCGTGACCGTGTCGTGGAACAGCGGCGCGC
TGACGAGCGGGGTCCACACCTTCCCGGCCGTGCTGCAGAGCAGCGGCCTCTACTCGCT
GAGCAGCGTGGTCACCGTGCCCAGCAGCAGCCTGGGGACCCAGACGTACATCTGCAAC
GTGAACCACAAGCCCTCGAACACCAAGGTCGACAAGAAGGTGGAGCCCCCGAAGAGCT
GCGACAAGACCCACACCTGCCCGCCCTGCCCCGCCCCCGAGCTCCTGGGCGGGCCCAG
CGTGTTCCTGTTCCCGCCCAAGCCCAAGGACACGCTCATGATCAGCCGCACCCCCGAG
GTCACCTGCGTGGTGGTCGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGT
ACGTCGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCGCGGGAGGAGCAGTACAA
CTCGACGTACCGCGTCGTGAGCGTGCTGACCGTCCTGCACCAGGACTGGCTCAACGGC
AAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCGCCCATCGAGAAGACCA
TCAGCAAGGCCAAGGGGCAGCCCCGGGAGCCGCAGGTGTACACCCTGCCCCCCAGCCG
CGACGAGCTCACGAAGAACCAGGTCAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCC
TCGGACATCGCCGTGGAGTGGGAGAGCAACGGGCAGCCGGAGAACAACTACAAGACCA
CCCCGCCCGTCCTCGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACGGTGGA
CAAGTCGCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTCATGCACGAGGCCCTC
CACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGGAAGCATCATCATCATC
ATCATTGACC**AGATCTTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGA**
CATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATATGCATACC***A***
TGGCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGAC
GCAGACCTGGGCCGGGGACATCCAGATGACCCAGAGCCCGTCGAGCCTGAGCGCCAGC
GTGGGCGACCGGGTCACGATCACCTGCCGCGCGAGCCAGGACGTGAACACCGCCGTGG
CCTGGTACCAGCAGAAGCCCGGGAAGGCCCCCAAGCTCCTGATCTACTCGGCGAGCTT
CCTGTACAGCGGCGTCCCCAGCCGGTTCAGCGGGTCGCGCAGCGGCACCGACTTCACG
CTCACCATCAGCAGCCTGCAGCCGGAGGACTTCGCCACCTACTACTGCCAGCAGCACT
ACACCACGCCCCCCACCTTCGGGCAGGGCACCAAGGTGGAGATCAAGCGGACCGTGGC
CGCCCCCAGCGTCTTCATCTTCCCGCCCAGCGACGAGCAGCTGAAGTCGGGCACGGCC
AGCGTGGTGTGCCTCCTGAACAACTTCTACCCCCGCGAGGCGAAGGTCCAGTGGAAGG
TGGACAACGCCCTGCAGAGCGGGAACAGCCAGGAGAGCGTGACCGAGCAGGACTCGAA
GGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAGGCCGACTACGAGAAG
CACAAGGTCTACGCCTGCGAGGTGACCCACCAGGGGCTCTCGAGCCCCGTGACCAAGA
GCTTCAACCGGGGCGAGTGCTGATGACCACTAG

Fig. 22

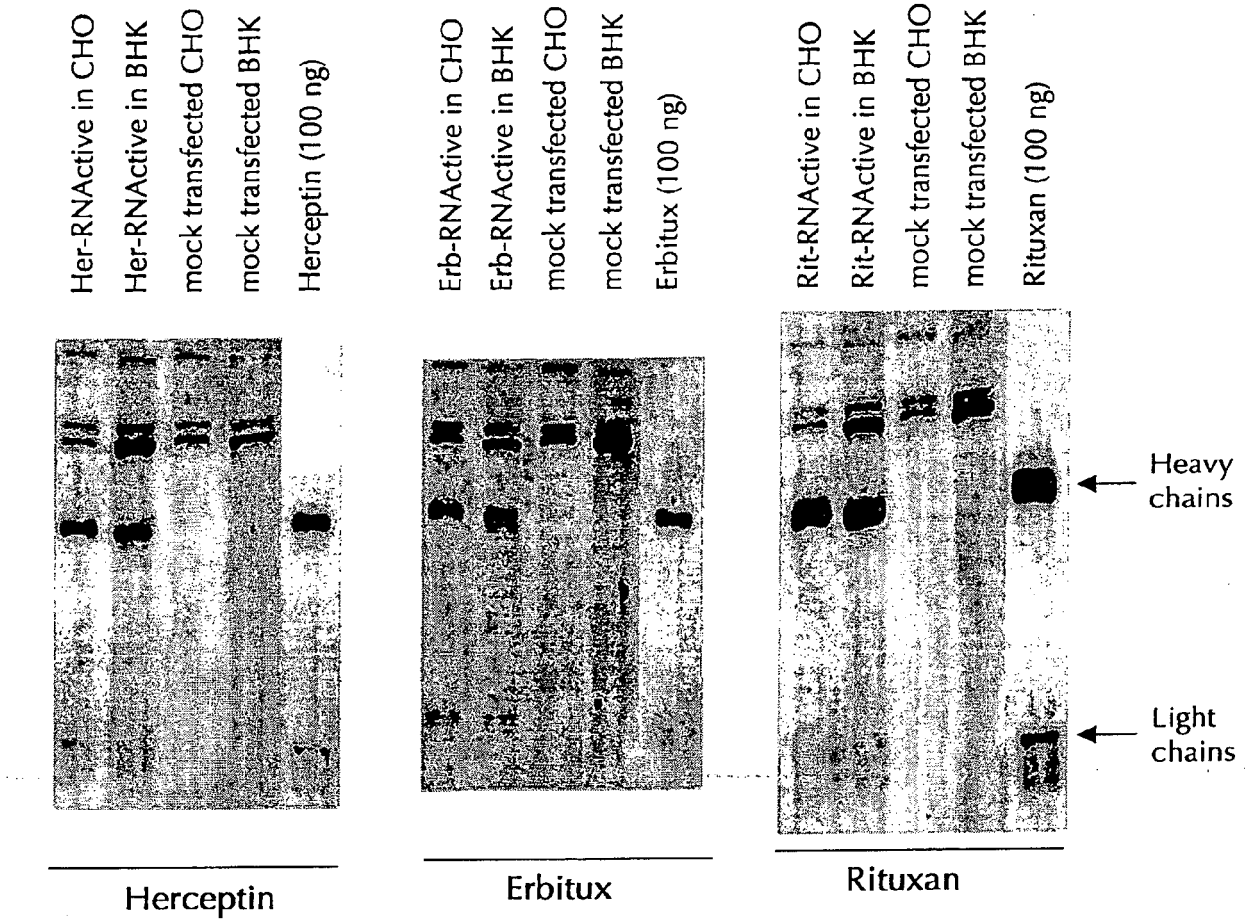
Her-RNActive in CHO
Her-RNActive in BHK
mock transfected CHO
mock transfected BHK
Herceptin (100 ng)
Erb-RNActive in CHO
Erb-RNActive in BHK
mock transfected CHO
mock transfected BHK
Erbitux (100 ng)
Rit-RNActive in CHO
Rit-RNActive in BHK
mock transfected CHO
mock transfected BHK
Rituxan (100 ng)
Heavy chains
Light chains
Herceptin
Erbitux
Rituxan

Fig. 23

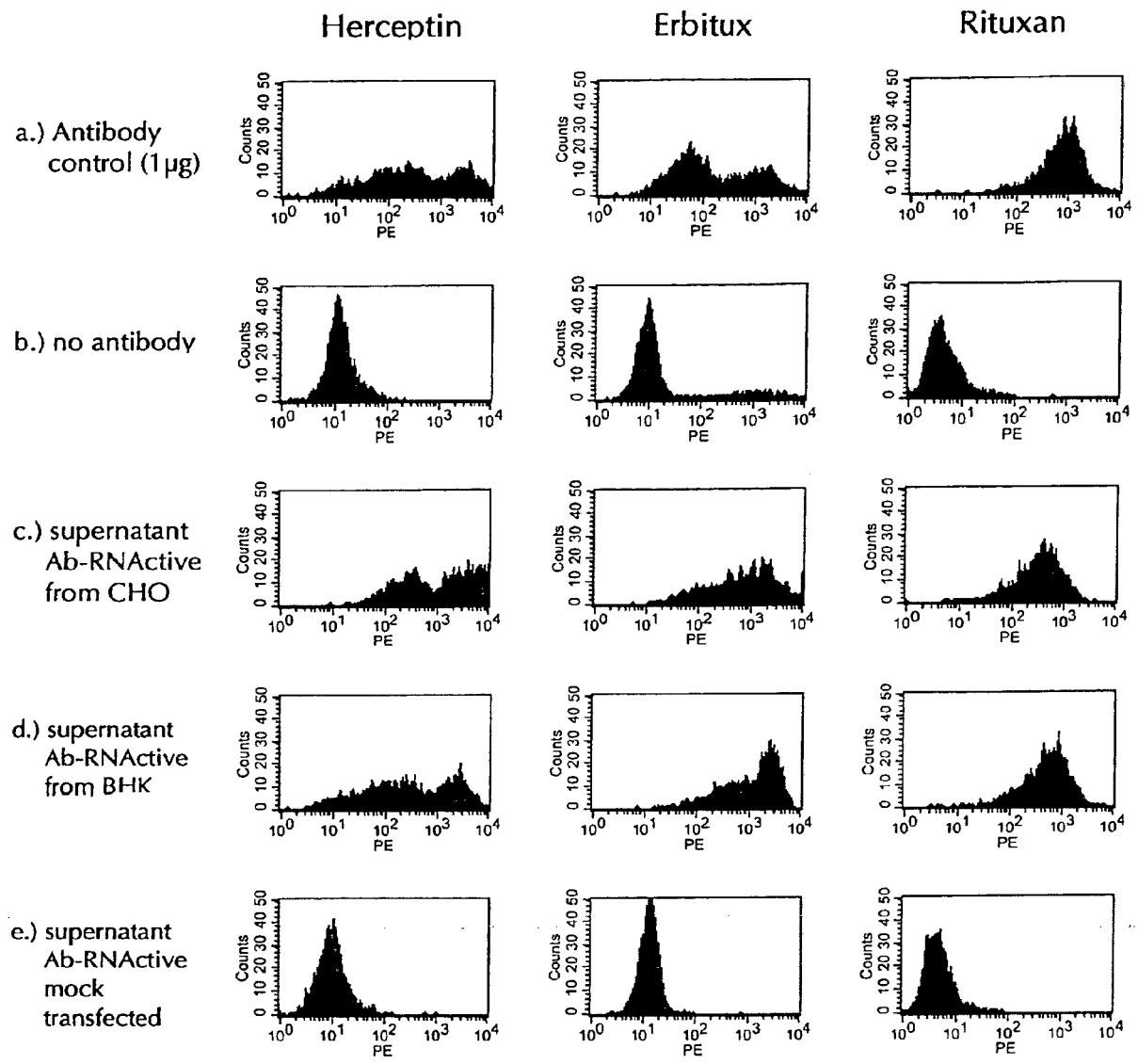
Herceptin
Erbitux
Rituxan
a.) Antibody control (1µg)
b.) no antibody
c.) supernatant Ab-RNActive from CHO
d.) supernatant Ab-RNActive from BHK
e.) supernatant Ab-RNActive mock transfected
Counts
PE

Fig. 24

AAGCTTACC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCC
TCGCCCTGACGCAGACCTGGGCCGGGCAGGCCTACCTGCAGCAGAGCGGCGCGGAGCT
CGTGCGGCCGGGGGCCTCGGTCAAGATGAGCTGCAAGGCCAGCGGCTACACCTTCACG
AGCTACAACATGCACTGGGTGAAGCAGACCCCGCGCCAGGGGCTGGAGTGGATCGGCG
CCATCTACCCCGGGAACGGCGACACCAGCTACAACCAGAAGTTCAAGGGCAAGGCGAC
CCTGACGGTGGACAAGTCGAGCAGCACCGCCTACATGCAGCTCAGCAGCCTGACCTCG
GAGGACAGCGCCGTCTACTTCTGCGCCCGGGTGGTGTACTACAGCAACAGCTACTGGT
ACTTCGACGTCTGGGGGACCGGCACGACCGTGACCGTGAGCGGGCCCAGCGTCTTCCC
CCTGGCCCCCTCGAGCAAGAGCACCAGCGGCGGCACGGCGGCCCTCGGGTGCCTGGTG
AAGGACTACTTCCCCGAGCCCGTGACCGTCAGCTGGAACTCGGGCGCCCTGACCAGCG
GGGTGCACACCTTCCCGGCCGTGCTCCAGAGCAGCGGCCTGTACAGCCTGAGCTCGGT
CGTGACGGTGCCCAGCAGCAGCCTCGGGACCCAGACCTACATCTGCAACGTCAACCAC
AAGCCCAGCAACACCAAGGTGGACAAGAAGGCGGAGCCCAAGTCGTGCGACAAGACGC
ACACCTGCCCGCCCTGCCCCGCCCCCGAGCTGCTGGGCGGCCCGAGCGTGTTCCTCTT
CCCGCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCCGAGGTCACGTGCGTG
GTGGTCGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTCGACGGGG
TGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTACAACAGCACCTACCG
CGTCGTGAGCGTGCTGACCGTCCTCCACCAGGACTGGCTGAACGGCAAGGAGTACAAG
TGCAAGGTGTCGAACAAGGCCCTGCCGGCCCCCATCGAGAAGACGATCAGCAAGGCGA
AGGGGCAGCCCCGGGAGCCCCAGGTGTACACCCTCCCGCCCAGCCGCGACGAGCTGAC
CAAGAACCAGGTCAGCCTGACCTGCCTCGTGAAGGGCTTCTACCCCAGCGACATCGCC
GTGGAGTGGGAGTCGAACGGGCAGCCCGAGAACAACTACAAGACGACCCCGCCCGTCC
TGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTCACCGTGGACAAGAGCCGGTG
GCAGCAGGGCAACGTGTTCAGCTGCTCGGTCATGCACGAGGCCCTGCACAACCACTAC
ACCCAGAAGAGCCTGAGCCTCAGCCCCGGGAAGCATCATCATCATCATCATTGACC**AT**
GCATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACAT
TTCTGACATTTCTGACATTTCTGACATTTCTGACATAGATCTACC***ATG***GCCGTGATGG
CGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGACGCAGACCTGGGC
CGGGCAGATCGTGCTGAGCCAGTCGCCGGCCATCCTCAGCGCGAGCCCCGGCGAGAAG
GTCACCATGACGTGCCGGGCCAGCAGCTCGGTGAGCTACATGCACTGGTACCAGCAGA
AGCCCGGGAGCAGCCCCAAGCCGTGGATCTACGCCCCCAGCAACCTGGCCTCGGGCGT
GCCCGCGCGCTTCAGCGGGAGCGGCAGCGGGACCAGCTACAGCCTGACCATCTCGCGG
GTCGAGGCCGAGGACGCCGCCACCTACTACTGCCAGCAGTGGAGCTTCAACCCGCCCA
CGTTCGGCGCCGGCACCAAGCTCGAGCTGAAGCGCACCGTGGCGGCCCCCAGCGTGTT
CATCTTCCCGCCCAGCGACGAGCAGCTGAAGAGCGGGACCGCCTCGGTCGTGTGCCTC
CTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTCGACAACGCGCTGC
AGAGCGGCAACAGCCAGGAGAGCGTGACGGAGCAGGACAGCAAGGACAGCACCTACTC
GCTCAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCC
TGCGAGGTCACGCACCAGGGGCTCAGCTCGCCCGTGACCAAGAGCTTCAACCGCTGAC
CACTAGT

Fig. 25

AAGCTTACC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCC
TCGCCCTGACGCAGACCTGGGCCGGGCAGGTGCAGCTGAAGCAGAGCGGCCCGGGGCT
CGTCCAGCCCTCGCAGAGCCTGAGCATCACCTGCACGGTGAGCGGCTTCAGCCTGACC
AACTACGGGGTGCACTGGGTCCGGCAGTCGCCCGGCAAGGGGCTCGAGTGGCTGGGCG
TGATCTGGAGCGGCGGGAACACCGACTACAACACCCCCTTCACGAGCCGCCTGAGCAT
CAACAAGGACAACAGCAAGTCGCAGGTGTTCTTCAAGATGAACAGCCTCCAGAGCAAC
GACACCGCCATCTACTACTGCGCGCGGGCCCTGACCTACTACGACTACGAGTTCGCCT
ACTGGGGCCAGGGGACCCTGGTCACGGTGAGCGCCGCGAGCACCAAGGGCCCGAGCGT
GTTCCCCCTCGCCCCCTCGAGCAAGAGCACCAGCGGCGGGACCGCCGCCCTGGGCTGC
CTGGTCAAGGACTACTTCCCCGAGCCGGTGACGGTGAGCTGGAACTCGGGGGCCCTCA
CCAGCGGCGTCCACACCTTCCCCGCGGTGCTGCAGAGCAGCGGGCTGTACAGCCTCAG
CTCGGTGGTCACCGTGCCCAGCAGCAGCCTGGGCACGCAGACCTACATCTGCAACGTG
AACCACAAGCCCAGCAACACCAAGGTCGACAAGCGCGTGGAGCCGAAGTCGCCCAAGA
GCTGCGACAAGACCCACACGTGCCCGCCCTGCCCCGCCCCCGAGCTGCTCGGCGGGCC
CAGCGTGTTCCTGTTCCCGCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCC
GAGGTCACCTGCGTGGTGGTCGACGTGAGCCACGAGGACCCGGAGGTGAAGTTCAACT
GGTACGTCGACGGCGTGGAGGTGCACAACGCCAAGACGAAGCCCCGCGAGGAGCAGTA
CAACAGCACCTACCGGGTCGTGTCGGTGCTCACCGTCCTGCACCAGGACTGGCTGAAC
GGGAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTCCCCGCGCCCATCGAGAAGA
CCATCAGCAAGGCCAAGGGCCAGCCGCGCGAGCCCCAGGTGTACACGCTGCCCCCCAG
CCGGGACGAGCTGACCAAGAACCAGGTCAGCCTCACCTGCCTGGTGAAGGGGTTCTAC
CCGTCGGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGA
CCACGCCCCCGGTCCTGGACAGCGACGGCAGCTTCTTCCTCTACAGCAAGCTGACCGT
GGACAAGAGCCGCTGGCAGCAGGGGAACGTGTTCTCGTGCAGCGTCATGCACGAGGCC
CTGCACAACCACTACACCCAGAAGAGCCTCAGCCTGAGCCCCGGCAAGCATCATCATC
ATCATCATTGACC**ATGCATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTC**
TGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATAGATCTA
CC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCT
GACGCAGACCTGGGCCGGGGACATCCTGCTCACCCAGAGCCCGGTGATCCTGTCGGTC
AGCCCCGGCGAGCGGGTGAGCTTCAGCTGCCGCGCCAGCCAGTCGATCGGGACGAACA
TCCACTGGTACCAGCAGCGGACCAACGGCAGCCCCCGCCTGCTCATCAAGTACGCGAG
CGAGAGCATCAGCGGGATCCCCTCGCGGTTCAGCGGCAGCGGGAGCGGCACCGACTTC
ACCCTGAGCATCAACAGCGTGGAGTCGGAGGACATCGCCGACTACTACTGCCAGCAGA
ACAACAACTGGCCGACGACCTTCGGCGCCGGGACCAAGCTGGAGCTCAAGCGCACCGT
CGCCGCGCCCAGCGTGTTCATCTTCCCGCCCAGCGACGAGCAGCTGAAGAGCGGCACG
GCCAGCGTGGTCTGCCTGCTCAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGA
AGGTGGACAACGCCCTGCAGTCGGGGAACAGCCAGGAGAGCGTCACCGAGCAGGACAG
CAAGGACAGCACCTACAGCCTGTCGAGCACCCTCACGCTGAGCAAGGCCGACTACGAG
AAGCACAAGGTGTACGCGTGCGAGGTGACCCACCAGGGCCTGAGCAGCCCCGTCACCA
AGTCGTTCAACCGCGGCGCCTGACCACTAGT

Fig. 26

AAGCTTACC***ATG***GCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCC
TCGCCCTGACGCAGACCTGGGCCGGGGAGGTGCAGCTGGTCGAGAGCGGCGGGGGCCT
CGTGCAGCCGGGCGGGTCGCTGCGGCTGAGCTGCGCCGCGAGCGGGTTCAACATCAAG
GACACCTACATCCACTGGGTGCGCCAGGCCCCCGGCAAGGGCCTCGAGTGGGTCGCCC
GGATCTACCCCACGAACGGGTACACCCGCTACGCCGACAGCGTGAAGGGCCGGTTCAC
CATCAGCGCGGACACCTCGAAGAACACGGCCTACCTGCAGATGAACAGCCTGCGCGCC
GAGGACACCGCCGTGTACTACTGCAGCCGGTGGGGCGGCGACGGGTTCTACGCCATGG
ACTACTGGGGGCAGGGCACCCTCGTCACCGTGAGCAGCGCGTCGACGAAGGGGCCCAG
CGTGTTCCCGCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGGACCGCCGCCCTGGGC
TGCCTCGTCAAGGACTACTTCCCCGAGCCCGTGACCGTGTCGTGGAACAGCGGCGCGC
TGACGAGCGGGGTCCACACCTTCCCGGCCGTGCTGCAGAGCAGCGGCCTCTACTCGCT
GAGCAGCGTGGTCACCGTGCCCAGCAGCAGCCTGGGGACCCAGACGTACATCTGCAAC
GTGAACCACAAGCCCTCGAACACCAAGGTCGACAAGAAGGTGGAGCCCCCGAAGAGCT
GCGACAAGACCCACACCTGCCCGCCCTGCCCCGCCCCCGAGCTCCTGGGCGGGCCCAG
CGTGTTCCTGTTCCCGCCCAAGCCCAAGGACACGCTCATGATCAGCCGCACCCCCGAG
GTCACCTGCGTGGTGGTCGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGT
ACGTCGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCGCGGGAGGAGCAGTACAA
CTCGACGTACCGCGTCGTGAGCGTGCTGACCGTCCTGCACCAGGACTGGCTCAACGGC
AAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCGCCCATCGAGAAGACCA
TCAGCAAGGCCAAGGGGCAGCCCCGGGAGCCGCAGGTGTACACCCTGCCCCCCAGCCG
CGACGAGCTCACGAAGAACCAGGTCAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCC
TCGGACATCGCCGTGGAGTGGGAGAGCAACGGGCAGCCGGAGAACAACTACAAGACCA
CCCCGCCCGTCCTCGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTGACGGTGGA
CAAGTCGCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTCATGCACGAGGCCCTC
CACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGGAAGCATCATCATCATC
ATCATTGACC**ATGCATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGA**
CATTTCTGACATTTCTGACATTTCTGACATTTCTGACATTTCTGACATAGATCTACC***A***
TGGCCGTGATGGCGCCGCGGACCCTGGTCCTCCTGCTGAGCGGCGCCCTCGCCCTGAC
GCAGACCTGGGCCGGGGACATCCAGATGACCCAGAGCCCGTCGAGCCTGAGCGCCAGC
GTGGGCGACCGGGTCACGATCACCTGCCGCGCGAGCCAGGACGTGAACACCGCCGTGG
CCTGGTACCAGCAGAAGCCCGGGAAGGCCCCCAAGCTCCTGATCTACTCGGCGAGCTT
CCTGTACAGCGGCGTCCCCAGCCGGTTCAGCGGGTCGCGCAGCGGCACCGACTTCACG
CTCACCATCAGCAGCCTGCAGCCGGAGGACTTCGCCACCTACTACTGCCAGCAGCACT
ACACCACGCCCCCCACCTTCGGGCAGGGCACCAAGGTGGAGATCAAGCGGACCGTGGC
CGCCCCCAGCGTCTTCATCTTCCCGCCCAGCGACGAGCAGCTGAAGTCGGGCACGGCC
AGCGTGGTGTGCCTCCTGAACAACTTCTACCCCCGCGAGGCGAAGGTCCAGTGGAAGG
TGGACAACGCCCTGCAGAGCGGGAACAGCCAGGAGAGCGTGACCGAGCAGGACTCGAA
GGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAGGCCGACTACGAGAAG
CACAAGGTCTACGCCTGCGAGGTGACCCACCAGGGGCTCTCGAGCCCCGTGACCAAGA
GCTTCAACCGGGGCGAGTGCTGATGACCACTAG

Fig. 27

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European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 16 00 1778

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	HU ET AL: "Codon optimization, expression, and characterization of an internalizing anti-ErbB2 single-chain antibody in Pichia pastoris", PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, SAN DIEGO, CA, vol. 47, no. 1, 1 May 2006 (2006-05-01), pages 249-257, XP005392638, ISSN: 1046-5928, DOI: 10.1016/J.PEP.2005.11.014 * page 252 *	1-6,18	INV. C07K16/28 A61K31/7088
X	MAYFIELD STEPHEN P ET AL: "Expression and assembly of a fully active antibody in algae", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 100, no. 2, 21 January 2003 (2003-01-21), pages 438-442, XP002440629, ISSN: 0027-8424, DOI: 10.1073/PNAS.0237108100 * pages 439-440 *	1-5,18	TECHNICAL FIELDS SEARCHED (IPC) A61K C07K
X	OHASHI ET AL: "Efficient protein selection based on ribosome display system with purified components", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ELSEVIER, AMSTERDAM, NL, vol. 352, no. 1, 30 November 2006 (2006-11-30), pages 270-276, XP005725399, ISSN: 0006-291X, DOI: 10.1016/J.BBRC.2006.11.017 * page 271, left-hand column *	1-5,18	

-/--

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	29 November 2016	Bumb, Peter

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 16 00 1778

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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Place of search	Date of completion of the search	Examiner
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